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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 48/00, 39/00, 38/00, 31/70, A01N 43/04, 63/00, G01N 33/574

A1

(11) International Publication Number: **WO 97/49432**

(43) International Publication Date: 31 December 1997 (31.12.97)

(21) International Application Number: PCT/US97/11210

(22) International Filing Date: 24 June 1997 (24.06.97)

(30) Priority Data:

08/669,676	24 June 1996 (24.06.96)	US
08/709,925	9 September 1996 (09.09.96)	US

(60) Parent Applications or Grants
(63) Related by Continuation

US	08/669,676 (CIP)
Filed on	24 June 1996 (24.06.96)
US	08/709,925 (CIP)
Filed on	9 September 1996 (09.09.96)

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(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, HU, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: TREATMENT AND PREVENTION OF CANCER BY ADMINISTRATION OF DERIVATIVES OF HUMAN CHORIONIC GONADOTROPIN

(57) Abstract

The present invention relates to methods of treating or preventing cancer by administration of human chorionic gonadotropin, β-human chorionic gonadotropin, a peptide containing a sequence of a portion of β-human chorionic gonadotropin, or a fraction of a source of human chorionic gonadotropin or β-human chorionic gonadotropin. In a preferred embodiment, the invention provides methods of treating or preventing Kaposi's Sarcoma, breast cancer, lung cancer or prostate cancer. The invention further provides assays for the utility of particular human chorionic gonadotropin preparations in the treatment or prevention of cancer. Pharmaceutical compositions and methods of administration are also provided.

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TREATMENT AND PREVENTION OF CANCER BY ADMINISTRATION OF DERIVATIVES OF HUMAN CHORIONIC GONADOTROPIN

1. CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation in part of co-pending application Serial No. 08/709,925, filed September 9, 1996, which is a continuation in part of co-pending application Serial No. 08/669,676, filed June 24, 1996, both of which are incorporated by reference herein in their entireties.

2. FIELD OF THE INVENTION

The present invention is directed to methods of treatment and prevention of cancer by administration of human chorionic gonadotropin preparations, fractions of native human chorionic gonadotropin preparations, the β-chain of human chorionic gonadotropin and peptides containing a sequence of one or more portions of the β-chain of human chorionic gonadotropin. The invention also provides pharmaceutical compositions comprising human chorionic gonadotropin preparations, fractions of human chorionic gonadotropin preparations, the β-chain of human chorionic gonadotropin or peptides having a sequence of one or more portions of the β-chain of human chorionic gonadotropin.

3. BACKGROUND OF THE INVENTION

3.1. CANCER

A neoplasm, or tumor, is a neoplastic mass resulting from abnormal uncontrolled cell growth, which may cause swelling on the body surface, and which can be benign or malignant. Benign tumors generally remain localized. Malignant tumors are collectively termed cancers. The term "malignant" generally means that the tumor can invade and destroy neighboring body structures and spread to distant sites to cause death (for review, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-122). Treatment options, such as

surgery, chemotherapy and radiation treatment, are either ineffective or present serious side effects. Thus, there is a need for development of new drugs for the treatment of cancer.

Kaposi's Sarcoma (KS) is a rare type of cancer, the incidence of which is greatly increased in HIV infected people (Lunardi-Iskandar, Y., et al., 1995, *Nature* 375:64-68; Friedman-Kien, A.E., et al., 1981, *J. Am. Acad. Dermatol.* 5:468-473). The tumors appear to be comprised of hyperplastic cells derived from vascular endothelial cells (Nakamura, S., et al., 1988, *Science* 242:426-430; Ensoli, B., et al, 1989, *Science* 243:223-226; Salahuddin, S.Z., et al., 1988, *Science* 242:430-433; Masood, R., et al., 1994, *AIDS Res. Hum. Retroviruses* 10:969-976; Lunardi-Iskandar, Y., et al., 1995, *JNCI* 88:450-454). In some cases, neoplastic cells with chromosomal abnormalities are also present in the tumors (Lunardi-Iskandar, Y., et al., 1995, *JNCI* 87:974-981; Delli-Bovi, P., et al., 1986, *Cancer Res.* 46:6333-6338; Siegal, B., et al., 1990, *Cancer* 65:492-498; Yunis, J.J., 1983, *Science* 221:227-236; Popescu, N.C., et al., 1995, *JNCI* 88:450-454). Therapies for KS include radiotherapy, α-interferon and systemic chemotherapy (Chak, L.Y., et al., 1988, *J. Clin. Oncol.* 6:863-7; Evans, L.M., et al., 1991, *J. Immunother.* 10:39-50; Kovas, J., et al., 1990, *Ann. Intern. Med.* 112:812-21; Gelmann, E.D., et al., 1987, *Am. J. Med.* 82:456-62; Gill, P.S., et al., 1991, *Am. J. Med.* 90:427-33; Gill, P.S., et al., 1990; *Am. J. Clin. Oncol.* 13:315-9; Gill, P.S., et al., 1994, *AIDS* 8:1695-9). However, hematological and non-hematological toxicities limit the prolonged use of chemotherapy and α-interferon in conjunction with anti-retroviral agents commonly used in the treatment of AIDS (Kovas, J., et al., 1990, *Ann. Intern. Med.* 112:812-21; Gill, P.S., et al., 1991, *Am. J. Med.* 90:427-33; Gill, P.S., et al., 1994, *AIDS* 8:1695-9). Thus, new drugs, preferably drugs compatible with AIDS therapeutics, are needed for the treatment of KS.

3.2. HUMAN CHORIONIC GONADOTROPIN

Human chorionic gonadotropin (hCG), which is required for the maintenance of pregnancy, is a member of the glycoprotein hormone family. The glycoprotein hormones, which also include follicle-stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH), consist of two sub-units, α and β. These subunits are non-covalently linked to form a heterodimer, and heterodimer formation has been shown to be required for receptor binding. Within a particular species, the α-subunits are identical among the glycoprotein hormones while the β-subunits differ and determine the receptor binding specificity of the particular hormone (Kornyei, J.L., et al., 1993, *Biol. Reprod.* 49:1149). The β-subunits of the glycoprotein hormones exhibit a high degree of sequence similarity within the N-terminal 114 amino acids. LH is the most similar to hCG with 85% sequence homology within the first 114 amino acids, and both proteins bind the same receptor. hCG, however, contains a C-terminal extension not present in the other glycoprotein β-chains (Lapthorn, A.J., et al., 1994, *Science* 369:455-461).

From the three dimensional crystal structure of hCG, it was determined that hCG, like the growth factors nerve growth factor (NGF), transforming growth factor-β (TGF-β) and platelet-derived growth factor-β (PDGF-β), is a cysteine-knot glycoprotein. Proteins containing such a cysteine-knot motif have at least three disulfide bridges, two of which join adjacent anti-parallel strands of the peptide, thus, forming a ring, and one of which joins the peptide chain through the ring. Particular structures in the hCG β-chain include the determinant loop sequence (β93-100) which has been implicated in subunit association and the longest inter-cysteine loop (β38-57) which may play a role in receptor binding. Residues 47-53 appear to be exposed at the surface of this inter-cysteine loop (Lapthorn et al., 1994, *Nature* 369:455-461).

References by Bellet et al. (PCT Publication WO94/20859; Australian Patent Publication AU 94/62112; PCT Publication

WO95/12299) disclose the use of hCG or certain peptides thereof for immunotherapy treatment of cancers which secrete hCG or hCG fragments. None of these references, however, describe the administration of the β-hCG peptides of the present invention.

Triozzi et al. (1994, *Int. J. Oncology* 5:1447-1453) describes the use of a synthetic β-hCG vaccine for treatment of a variety of non-trophoblastic cancers by production of an immune response against a portion of hCG. The only vaccine disclosed in the reference consists of the carboxy terminal peptide of β-hCG (amino acids 109-145) conjugated to diphtheria toxoid. Furthermore, Stevens (U.S. Patent 4,691,006) used modified peptides for treatment of, *inter alia*, hormone-related diseases and disorders and hormone-associated carcinomas. C-terminal β-hCG peptides, *i.e.*, peptides and subpeptides of amino acids 111-145, which have been modified in some way are described for use in treating hCG secreting cancers. The patent specifically indicates that peptides from amino acids 1-110 of β-hCG are not useful for treating such cancers.

Lunardi-Iskandar et al. (1995, *Nature* 375:64-68 and PCT Publication WO96/04008) discloses that hCG, β-hCG, as well as β-hCG carboxy-terminal peptides of amino acids 109-145 (SEQ ID NO:25) and 109-119 (SEQ ID NO:7) are useful for treatment of Kaposi's Sarcoma. Phase II clinical trials have been carried out in which intralesional injections with some commercial preparations of hCG resulted in remissions of KS lesions (Gill, P.S., et al., 1996, *New Eng. J. Med.*, submitted) and systemic injection resulted in regression of far advanced pulmonary KS in two patients (Hermans, P., et al., 1995, *AIDS Res. Hum. Retroviruses* S:96). Harris (1995, *The Lancet* 346:118-119) reported that high doses of hCG, specifically 150,000 IU to 700,000 IU three times per week intramuscularly, but not doses of 100,000 IU three times per week given intramuscularly lead to KS tumor regression in certain KS patients. However, none of these references suggests using the peptides of the present invention.

Citation of references hereinabove shall not be construed as an admission that such references are prior art to the present invention.

4. SUMMARY OF THE INVENTION

The present invention further relates to therapeutic methods and compositions for treatment and prevention of cancers based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG and therapeutically and prophylactically effective proteins containing a sequence of one or more portions (*i.e.*, a fusion protein comprising more than one β-hCG peptide sequence either as non-contiguous or contiguous sequences, *e.g.*, having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide) of β-hCG, and related derivatives and analogs. The invention provides for treatment and prevention of cancers by administration of a therapeutic compound of the invention. The therapeutic compounds of the invention include: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, therapeutically and prophylactically effective peptides having a sequence of one or more portions of β-hCG, modified derivatives of hCG, β-hCG and β-hCG peptides, and nucleic acids encoding β-hCG and therapeutically and prophylactically effective peptides having a sequence of one or more portions of β-hCG, and derivatives and analogs of the foregoing.

The present invention also relates to certain fractions (*i.e.* components of a source of hCG or β-hCG isolated away from other components in the source of hCG or β-hCG by a separation technique known in the art) of any source of hCG or β-hCG, such as commercial hCG preparations and human (preferably early, *i.e.*, first trimester) pregnancy urine, which fractions have anti-HIV and or anti-Kaposi's Sarcoma activity. The invention also provides *in vitro* and *in vivo* assays for assessing the efficacy of therapeutics of the invention for treatment or prevention of cancers.

The invention also provides pharmaceutical compositions and methods of administration of Therapeutics of the invention for treatment.

5. DESCRIPTION OF THE FIGURES

Figures 1A-J. Effects of administration of hCG preparations on HIV-1 viral load and CD4+ T cell levels in individual patients in the clinical study described in Section 7.1 *infra*. Figures A and B are data from patient PHOJ, C and D from patient PG1, E and F from patient PG3, G and H from patient PHVE, and I and J from patient PG17. In panels A, C, E, G and I, viral load and CD4+ T Cell counts are plotted over time (in months). Viral load (measured by RT-PCR in panels A and G and by the Roche Amplicor test in panels C, E and I) is plotted as the logarithm of the viral load (represented by line with "X" data points). The CD4+ T Cell levels are plotted as CD4+ T Cells/ml (represented by line with triangle data points). Panels B, D, F, H, and J plot the dosage of hCG in IU (X 1000) per week over time in months, with the timing of other therapies indicated above the graph with a thick arrow.

Figures 2A-H. Effect of hCG preparations and peptides on KS colony growth *in vitro* and KS tumors *in vivo*. (A) Comparison of the anti-KS *in vitro* effects (tumor cell killing) of purified hCG and β-hCG peptides in KS clonogenic assays using KS Y-12 and KS "SKL"18 cells depicted in a bar graph in terms of number of colonies. The results are averages of 3 sets of results with less than 10% variation and are representative of multiple experiments. Results with no hCG or hCG peptides are represented by open bars, the results with the β-hCG peptide of amino acids 109-119 (SEQ ID NO:7) are represented by stippled bars, the results with the β-hCG peptide of amino acids 109-145 (SEQ ID NO:25) are represented by the bars with horizontal stripes, the results with the circular β-hCG peptide of amino acids 44-57 (SEQ ID NO:12) where the amino acid at position 44 is a cysteine are represented by the bars with diagonal stripes, and the

results with the highly purified hCG preparation, CR 127, are represented by solid bars. (B-H) Thin sections of KS tumors induced in nude mice by inoculation with 10^6 neoplastic KS Y-1 cells. (B) Thin section of tumors from mice that were not treated with hCG or hCG subunits or peptides (C) Thin section of a tumor from a mouse after treatment with crude hCG APL (100 IU) subcutaneously daily for 7 days. (D) Thin section of a tumor from a mouse treated with the β-hCG peptide of amino acids 45-57 (SEQ ID NO:6), 10 μg/ml/daily (6.7 nmoles) for 5 days. (E) Thin section of a tumor from a mouse after 5 days of treatment with the circularized β-hCG peptide 44-57 (SEQ ID NO:12), where cysteine has been substituted at position 44, at 10 μg per day. (F) This panel shows the thin tissue section of KS tumor from AIDS-KS patients treated with 1 ml of diluent alone shows less than 2% cell death as detected by specific apoptosis in situ immunostaining. (G) Thin tissue section of KS tumor from an AIDS-KS patient after hCG preparation therapy of intralesional injections of 2000 IU, 3 times weekly for 2-3 weeks, shows evidence of apoptosis in all cells. (H) Thin tissue section of KS tumor from an AIDS-KS patient after hCG preparation therapy, 500 IU, 3 times weekly for 3 weeks.

Figure 3. The nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of β-hCG.

Figures 4A and B. Schematic depiction of the structures of (A) the linear peptide of amino acids 45-57 (SEQ ID NO:6) of the β-hCG sequence depicted in Figure 3 (SEQ ID NO:2) where the amino acid residues at positions 47 and 51 are substituted by a branch made up of diaminobutyric acid peptide bonded to proline, and (B) the circularized peptide of amino acids 44-57 (SEQ ID NO:12) with valine at position 44 substituted with cysteine, which protein is circularized via a disulfide bond between its amino- and carboxy-terminal cysteines. In both A and B, amino acids are represented by their three letter amino acid code, except for the branched residues and the terminal cysteines, for which the structure is depicted.

Figures 5A-F. These graphs depict results from the fractionation by SUPERDEX™ 200 gel filtration of a commercial hCG preparation APL™ (Wyeth Ayerst) and early pregnancy urine. (A) and (D). These graphs depicts the relative amount of protein in mg/ml in each fraction identified by fraction number in the hCG APL™ fractionation (A) and early pregnancy urine fractionation (D). The fractions containing the hCG dimer and β-core protein are identified with arrows and the labels "hCG" and "β-core" respectively. (B) and (E). These graphs present the percent inhibition of growth of cultured KS cells by the individual fractions from the hCG APL™ (B) and early pregnancy urine (E) using KS cell clonogenic assays. The results are plotted as percent inhibition versus fraction number. (C) and (F). These graphs depict the effect of the hCG APL™ (C) and early pregnancy urine (F) fractions on HIV replication in vitro. Specifically, this graph presents data on the percentage inhibition of HIV-1 IIIB viral infection of PBMCs as a function of fraction number.

Figure 6. Effect of hCG preparations, hCG and early pregnancy urine fractions, and β-hCG peptides on KS cell growth in vitro. Results for clonogenic assays using the cell lines KS Y-1 (bars labelled "KS Y-1") and KS SLK (labelled "KS SLK") are presented on a bar graph as percent inhibition of KS Colony Growth. Bars 1-4 represent cells treated with 200 IU/ml of the commercial hCG preparations hCG APL™, hCG CG10 (Sigma), hCG PROFASI™, and hCG PREGNYL™, respectively; bar 5 represents treatment with 50 μg/ml β-hCG core protein; bar 6, 50 μg/ml native β-hCG; bar 7, 50μg/ml native α-hCG; bar 8, 200 IU/ml highly purified hCG preparation CR 127; bar 9, 50 μg/ml recombinantly produced hCG (Sigma); bars 10 and 11, 50-100 μl/ml of fractions 65 and 76, respectively, of the early pregnancy urine fractionation; bars 12 and 13, 50-100 μl/ml of fractions 65 and 76, respectively, of the hCG APL™ fractionation; and bar 14, 100 μg/ml of the circularized β-hCG peptide 44-57 (with cysteine substituted at postion 44; SEQ ID NO:26).

Figure 7. Effect of treatment with hCG commercial preparation and early pregnancy urine fractions on KS tumors in mice. Results are plotted as percent inhibition of tumor size as compared to control tumors. The open bar represents mice treated with PBS alone; the solid bar with 100 IU per day of hCG APL™; diagonally striped bars with 200 µl per day of fractions 26, 76, 65, and 82 (as labelled on top of the bars) of the early pregnancy urine fractionation ("HAF-UF#"); and open bars with 200 µl per day of fractions 62, 65, 74, 76 and 35 (as labelled on top of the bars) of the hCG APL™ fractionation ("HAF-CF#").

Figures 8A-C. Graphs of change in viral load and $CD4^+$ T cell levels with hCG therapy. (A) The change in viral load is plotted as the logarithm of viral load after therapy ("Logload") as a function of viral load before therapy ("Baselog"). (B) The change in $CD4^+$ T cell levels is plotted as $CD4^+$ T cell levels after therapy (in $CD4^+$ T cells/ml) ("CD4") as a function of $CD4^+$ T cell levels before therapy (in $CD4^+$ T cells/ml) ("CD4Base"). (C) Plot of linear regression analysis of the change in viral load ("vlchange") as a function of weekly dose of hCG in IU ("HCGIU"). For all three panels, data points for patients on hCG therapy as well as non-protease and protease inhibitors are represented by open triangles, those on hCG therapy and non-protease inhibitors by open diamonds, and those on hCG alone by solid circles.

Figures 9A and B. (A) Plot of protein concentration (as mAUFS, milli absorbance units, at 280 nm) as a function of the fraction number of the hCG APL™ preparation SUPERDEX™ 200 fractionation. (B) Plot of protein concentration (as mAUFS, milli absorbance units, at 280 nm) of molecular weight markers of 670 kD, 158 kD, 44 kD, 17 kD and 1.3 kD (as indicated above the plot) as a function of fraction number of a SUPERDEX™ 200 column run under the same conditions as the fractionation plotted in panel A.

Figures 10A-E. Mass spectrometry profiles of fractions 61, 63, 64, 65, and 67 in panels A-E, respectively.

Figures 11 A-H. Visualization by confocal microscopy of apoptosis of prostate cancer cells treated with hCG and hCG related preparations for 48 hours and stained with both actin monoclonal antibody labelled with FITC and propidium iodine. Panel A shows a confocal micrograph of cultured prostate tumor cells untreated; Panel B, prostate tumor cells treated with 200 IU hCG APL™; Panel C, prostate tumor cells treated with 200 μg of the circularized β-hCG peptide of amino acids 44-57 with cysteine substituted at position 44 (SEQ ID NO:26); Panel D, prostate tumor cells treated with 100 μl of fraction number 64 of the early pregnancy urine; Panels E-H are controls treated with PBS alone.

Figure 12. Results of clonogenic assays on prostate tumor cells with hCG preparations and fractions of the hCG APL™ preparations. Results are plotted both as number of colonies (bars) and percent inhibition of colony formation (line with solid diamonds) for each test substance. "PBS" are cells treated with PBS alone; "apl100" and "apl200" are cells treated with 100 IU and 200 IU of the hCG APL™ preparation, respectively; the numbers are cells treated with those fraction numbers of the fractionation of the hCG APL™ preparation; "alpha" are those cells treated with a native α-hCG preparation; and "apl-d" are those cells treated with the hCG APL™ diluent alone (*i.e.*, a control without hCG).

Figures 13A-C. Photographs showing the levels of apoptosis in tumor cells in nude mice in response to treatment with hCG preparations or β-hCG peptides. Panel A presents tumor cells from untreated mice. Panel B presents tumor cells from mice treated with 100 μg/day of the circularized β-hCG peptide of amino acids 44-57 with cysteine substituted at position 44 (SEQ ID NO:26). Panel C presents tumor cells from mice treated with 100 IU per day of hCG APL™.

Figure 14. Bar graph depicting the percent of dead lung cancer cells as measured by Trypan Blue assay in response to hCG preparations, hCG fractions, and β-hCG peptides. Treatments are indicated as follows: "neg/pbs100" is control

treated with PBS alone; "apl/200" and "apl/500" were treated with 200 IU and 500 IU, respectively, of the hCG APL™ preparation; "sata2/200" and "sata2/300" were treated with 200 μg/ml and 300 μg/ml, respectively, of the circularized β-hCG peptide of amino acids 44-57, with cysteine substituted for position 44 (SEQ ID NO:26); "uf-60/200", "uf-64/200", "uf-74/200", "uf-23/200" and "uf-80/200" were treated with 200 μg/ml of the early pregnancy urine SUPERDEX™ 200 fractions 60, 64, 74, 23, and 80, respectively; "apl-64/200", "apl-65/200", "apl-67/200", "apl-72/200", "apl-74/200" and "apl-75/200" were treated with 200 μg/ml of the hCG APL™ SUPERDEX™ 200 fractions 64, 65, 67, 72, 74, and 75, respectively.

Figures 15A-C. Results of clonogenic assays on lung tumor cells with hCG preparations, fractions of hCG preparations, and β-hCG peptides. Results are plotted both as number of colonies (bars) and percent inhibition of colony formation (line with solid diamonds) for each test substance. (A) Treatments are indicated as follows: "100ul PBS" is control treated with PBS alone; "APL100" and "APL200" were treated with 100 IU and 200 IU, respectively, of the hCG APL™ preparation; "SATA2100" and "SATA2200" were treated with 100 μg/ml and 200 μg/ml, respectively, of the circularized β-hCG peptide of amino acids 44-57, with cysteine substituted for position 44 (SEQ ID NO:26); "uf-60/200", "uf-64/200", and "uf-74/200" were treated with 200 μg/ml of the early pregnancy urine SUPERDEX™ 200 fractions 60, 64, and 74, respectively; "apl-60/200", "apl-64/200" and "apl-74/200" were treated with 200 μg/ml of the hCG APL™ SUPERDEX™ 200 fractions 60, 64, and 74, respectively. (B) Results after 6 days of treatment. Treatments are indicated as follows: "100ul PBS" is control treated with PBS alone; "APL100" and was treated with 100 IU of the hCG APL™ preparation; "SATA1100" was treated with 100 μg/ml of the β-hCG peptide of amino acids 45-57 (SEQ ID NO: 6); "SATA2100" was treated with 100 μg/ml of the circularized β-hCG peptide of amino acids 44-57, with cysteine substituted for position 44 (SEQ ID

NO:26); and "SATAB100" was treated with 100 μg/ml of the fused β-hCG peptide 45-57::109-119 (SEQ ID NO:30). (C) Results after 5 days of treatment. Treatments are indicated as follows: "100ul PBS" is control treated with PBS alone; "APL100" and "APL200" were treated with 100 IU and 200 IU, respectively, of the hCG APL™ preparation; "SATA2100" and "SATA2200" were treated with 100 μg/ml and 200 μg/ml, respectively, of the circularized β-hCG peptide of amino acids 44-57, with cysteine substituted for position 44 (SEQ ID NO:26); "uf-60/200", "uf-64/200", and "uf-74/200" were treated with 200 μg/ml of the early pregnancy urine SUPERDEX™ 200 fractions 60, 64, and 74, respectively; "apl-60/200", "apl-64/200" and "apl-74/200" were treated with 200 μg/ml of the hCG APL™ SUPERDEX™ 200 fractions 60, 64, and 74, respectively.

Figures 16 A-H. Visualization by confocal microscopy of apoptosis of lung cancer cells treated with hCG and hCG related preparations for 48 hours and stained with both actin monoclonal antibody labelled with FITC and propidium iodine. Panel A shows a confocal micrograph of cultured lung tumor cells untreated; Panel B, lung tumor cells treated with 200 IU hCG APL™; Panel C, lung tumor cells treated with 200 μg of the circularized β-hCG peptide of amino acids 44-57 with cysteine substituted at position 44 (SEQ ID NO:26); Panel D, lung tumor cells treated with fraction number 64 of the early pregnancy urine; Panels E-H are controls treated with PBS alone.

Figures 17 A-I. Visualization by confocal microscopy of apoptosis of kidney (renal) cancer cells treated with hCG APL™ preparation and stained with both actin monoclonal antibody labelled with FITC and propidium iodine. Panels A-C show a confocal micrograph of cultured kidney cancer cells treated with PBS alone; Panels D-F, cultured kidney cancer cells treated for 48 hours with 100 IU hCG APL™; Panels G-I, cultured kidney cancer cells treated with 300 IU hCG APL™ for 48 hours.

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6. DETAILED DESCRIPTION OF THE INVENTION

The present invention further relates to therapeutic methods and compositions for treatment and prevention of cancers based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG, and therapeutically and prophylactically effective β-hCG peptides. The invention provides for treatment of cancer by administration of a therapeutic compound (termed herein "Therapeutic") of the invention. Such Therapeutics of the invention include but are not limited to: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, therapeutically and prophylactically effective β-hCG proteins (*i.e.*, those peptides which prevent or treat cancer), related derivatives and analogs of hCG, β-hCG or β-hCG peptides, and nucleic acids encoding β-hCG and β-hCG peptides, and analogs and derivatives thereof. β-hCG peptides and fractions of a source of native hCG or β-hCG which are effective for treatment and prevention of cancer can be identified by *in vitro* and *in vivo* assays such as those described in Section 6.2, *infra*.

In a preferred embodiment, a therapeutic composition of the invention comprises a β-hCG peptide, the amino acid sequence of which consists of amino acid numbers 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of Figure 3 (a portion of SEQ ID NO:2), particularly a β-hCG peptide which consists of amino acid numbers 41-54, 45-54 or 109-119 (SEQ ID NOS:3, 4, or 7, respectively), most preferably of a β-hCG peptide which consists of amino acid numbers 47-53 (SEQ ID NO:5) or 45-57 (SEQ ID NO:6). In another preferred embodiment, a therapeutic composition of the invention comprises a fusion protein comprising more than one β-hCG sequence (preferably non-contiguous sequences), *e.g.* having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-

hCG peptide, in particular a protein, the amino acid sequence of which consists of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an isolated protein of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 3 (portions of SEQ ID NO:2), *i.e.*, the peptides denoted 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively).

In other preferred embodiments, the therapeutic comprises a β-hCG peptide, the amino acid sequence of which consists of circularized (via a disulfide bond between its amino- and carboxy-terminal cysteines) 44-57 (SEQ ID NO:26) with the valine at position 44 substituted with cysteine ((Val44Cys) 45-57 circularized) (depicted in Figure 4B), the circularized (via a disulfide bond between its amino- and carboxy- terminal cysteines) fused peptide of amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6), or the peptide 45-57 (SEQ ID NO:6) where the amino acid residues at positions 47 and 51 are substituted by a branch, where the branches are made up of diaminobutyric acid peptide bonded to a proline residue (depicted in Figure 4A). The amino acid sequence of β-hCG is depicted in Figure 3 (SEQ ID NO:2).

In yet another embodiment, the therapeutic comprises fractions, preferably gel filtration fractions of a source of native hCG or native β-hCG (*i.e.* from or derived from a naturally occurring source of hCG or β-hCG and not recombinantly produced hCG or β-hCG), such as commercial hCG preparations and human pregnancy (preferably early, *i.e.* first trimester) urine, of material eluting from a SUPERDEX™ 200 (Pharmacia) gel filtration column with apparent molecular weights of approximately 40 kD, 15 kD or 2-3 kD as determined

based on in which fractions native hCG dimer (77kD) and β-hCG core (10 kD) elute.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

6.1. THERAPEUTIC USES

The invention provides for treatment or prevention of cancer by administration of a therapeutic compound (termed herein "Therapeutic"). Such "Therapeutics" include, but are not limited to: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, and therapeutically and prophylactically effective β-hCG peptides, *i.e.*, those fractions and peptides which prevent or treat HIV infection (*e.g.*, as demonstrated in *in vitro* and *in vivo* assays described *infra*), and derivatives and analogs thereof, as well as nucleic acids encoding hCG, β-hCG and therapeutically and prophylactically effective β-hCG peptides and derivatives and analogs thereof (*e.g.*, for use in gene therapy).

6.1.1. TREATMENT OF MALIGNANCIES

Malignancies and related disorders that can be treated or prevented by administration of a Therapeutic of the invention include, but are not limited to, those disorders listed in Table 1 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippencott Co., Philadelphia):

TABLE 1
MALIGNANCIES AND RELATED DISORDERS

Leukemia
- acute leukemia
  - acute lymphocytic leukemia
  - acute myelocytic leukemia
    - myeloblastic
    - promyelocytic
    - myelomonocytic
    - monocytic

erythroleukemia
chronic leukemia
chronic myelocytic (granulocytic) leukemia
chronic lymphocytic leukemia
Polycythemia vera
Lymphoma
Hodgkin's disease
non-Hodgkin's disease
Multiple myeloma
Waldenström's macroglobulinemia
Heavy chain disease
Solid tumors
sarcomas and carcinomas
fibrosarcoma
myxosarcoma
liposarcoma
chondrosarcoma
osteogenic sarcoma
chordoma
angiosarcoma
endotheliosarcoma
lymphangiosarcoma
Kaposi's sarcoma
lymphangioendotheliosarcoma
synovioma
mesothelioma
Ewing's tumor
leiomyosarcoma
rhabdomyosarcoma
colon carcinoma
pancreatic cancer
breast cancer
ovarian cancer
prostate cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma
bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
uterine cancer
testicular tumor
lung carcinoma

small cell lung carcinoma
bladder carcinoma
epithelial carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
meningioma
melanoma
neuroblastoma
retinoblastoma
Virally induced cancers

In specific embodiments, a Therapeutic of the invention is used to treat a neoplasm such as a gestational trophoblastic tumor, or testicular germ cell tumor, or cancer of the bladder, pancreas, cervix, lung, liver, ovary, colon or stomach, or adenocarcinoma or a virally induced cancer. In a preferred embodiment, a Therapeutic of the invention is used to treat neuroblastoma or carcinoma of the ovary or stomach. In a more preferred embodiment, a Therapeutic of the invention is used to treat Kaposi's sarcoma or carcinoma of the breast, lung, prostate, or kidney (renal).

In one aspect of the invention, the Therapeutic is administered in conjunction with other cancer therapy, such as chemotherapy (*e.g.*, treatment with adriamycin, bleomycin, vincristine, vinblastine, doxorubicin and/or Taxol).

The efficacy of a Therapeutic against a particular cancer can be determined by any method known in the art, for example but not limited to, those methods described in Section 6.2 *infra*.

6.1.1.1. PREMALIGNANT CONDITIONS

The Therapeutics of the invention can also be administered to treat premalignant conditions and to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 1. Such

prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79). Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a Therapeutic of the invention. Such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, etc..

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; *see* Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113, etc.)

In another specific embodiment, a Therapeutic of the invention is administered to a human patient to prevent progression to breast, colon, lung, pancreatic, or uterine cancer, or melanoma or sarcoma.

6.1.2. β-hCG PEPTIDES AND DERIVATIVES

In a preferred embodiment of the invention, proteins (*e.g.*, peptides), the amino acid sequence of which consists of one or more portions of the β-hCG sequence (β-hCG peptides) are used to treat or prevent cancer. In various specific embodiments, the portions of the β-hCG sequence are at least 3, 5, 10, 20, or 30 amino acids. These peptides are preferably β-hCG peptides, or nucleic acids encoding β-hCG peptides, from amino acids 41-54, 45-54, 47-53 and 45-57 (SEQ ID NOS:3-6, respectively) of Figure 3 (a portion of SEQ ID NO:2). In other embodiments, β-hCG peptides of 109-119, 41-

53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:7-25 and 33-35, respectively) of Figure 3 (a portion of SEQ ID NO:2) are used to treat or prevent cancer.

In another embodiment, the invention provides proteins, the amino acid sequences of which consist of two or more at least 5, 7 or 10 amino acid, non-naturally contiguous portions of the β-hCG sequence (Figure 3 (SEQ ID NO:2)) linked by peptide bonds between the N-terminus of one portion and the C-terminus of another portion. Specifically, proteins, the amino acid sequences of which consist of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of a peptide of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an isolated protein of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 3 (portions of SEQ ID NO:2), *i.e.*, the fused peptides denoted as 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). Derivatives of the foregoing fusion proteins are also provided (*e.g.*, branched, cyclized, N- or C-terminal chemically modified, etc.). In another embodiment, fusion proteins comprising two or more such portions of the β-hCG sequence are provided; such portions may or may not be contiguous to one another (*i.e.*, an intervening sequence may be present). Molecules comprising such portions linked by hydrocarbon linkages are also provided. In yet another embodiment a protein is used which is a cyclic, fused peptide, particularly a cyclic, fused peptide having a sequence consisting of β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and being circularized by a disulfide bond between the terminal cysteines at positions 110 and 57. In another embodiment,

the peptides of the invention (i) have an amino acid sequence consisting of no more than 8 peptides of the β-hCG sequence (Figure 3 (SEQ ID NO:2)) and (ii) comprise amino acid numbers 47-53 (SEQ ID NO:5) of β-hCG (Figure 3 (SEQ ID NO:2)).

In another embodiment, a protein is used that (a) comprises a β-hCG amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-119, 109-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-25 or 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2) and; (b) lacks β-hCG amino acids contiguous to said sequence. Peptides containing the above sequences in which only conservative substitutions have been made are also provided by the present invention, as but one example of peptide derivatives within the scope of the invention. Analogs of the above-mentioned proteins and peptides which have one or more amino acid substitutions forming a branched peptide (*e.g.*, by substitution with an amino acid or amino acid analog having a free amino- or carboxy-side chain that forms a peptide bond with a sequence of one or more amino acids, including but not limited to prolines) or allowing circularization of the peptide (*e.g.*, by substitution with a cysteine, or insertion of a cysteine at the amino- or carboxy-terminus or internally), to provide a sulfhydryl group for disulfide bond formation, are also provided.

Other β-hCG peptides and derivatives , and nucleic acids encoding these peptides and derivatives, may have utility in the therapeutic methods of the invention. The utility of β-hCG peptides may be determined by the *in vitro* and *in vivo* assays described in Section 6.2 *infra* or by any other method known in the art.

In specific embodiments, peptides of less than 50, or less than 25, amino acids are provided.

The invention also relates to derivatives, modifications and analogs of β-hCG peptides. In a specific embodiment, a purified derivative of a protein, the amino acid sequence of

which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-25 or 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2) is used to treat or prevent cancer. In one embodiment, β-hCG peptide derivatives can be made by altering the β-hCG peptide sequence by substitutions, additions or deletions that provide for therapeutically effective molecules. Thus, the β-hCG peptide derivatives include peptides containing, as a primary amino acid sequence, all or part of the particular β-hCG peptide sequence including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a peptide which is functionally active. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Conservative substitutions for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such β-hCG peptide derivatives can be made either by chemical peptide synthesis or by recombinant production from a nucleic acid encoding the β-hCG peptide which nucleic acid has been mutated. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical mutagenesis, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, *J. Biol. Chem* 253:6551), use of TAB® linkers (Pharmacia), etc.

In addition, β-hCG peptides and analogs and derivatives of β-hCG peptides can be chemically synthesized. (*See, e.g.*, Merrifield, 1963, *J. Amer. Chem. Soc.* 85:2149-2156.) For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (*e.g.*, *see* Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 50-60). β-hCG peptides can also be synthesized by use of a peptide synthesizer. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 34-49). Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the β-hCG peptide. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

By way of example but not by way of limitation, peptides of the invention can be chemically synthesized and purified as follows: Peptides can be synthesized by employing the N-α-9-fluorenylmethyloxycarbonyl or Fmoc solid phase peptide synthesis chemistry using a Rainin Symphony Multiplex Peptide Synthesizer. The standard cycle used for coupling of an amino acid to the peptide-resin growing chain generally includes: (1) washing the peptide-resin three times for 30 seconds with N,N-dimethylformamide (DMF); (2) removing the

Fmoc protective group on the amino terminus by deprotection with 20% piperidine in DMF by two washes for 15 minutes each, during which process mixing is effected by bubbling nitrogen through the reaction vessel for one second every 10 seconds to prevent peptide-resin settling; (3) washing the peptide-resin three times for 30 seconds with DMF; (4) coupling the amino acid to the peptide resin by addition of equal volumes of a 250 mM solution of the Fmoc derivative of the appropriate amino acid and an activator mix consisting or 400 mM N-methylmorpholine and 250 mM (2-(1H-benzotriazol-1-4))-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in DMF; (5) allowing the solution to mix for 45 minutes; and (6) washing the peptide-resin three times for 30 seconds of DMF. This cycle can be repeated as necessary with the appropriate amino acids in sequence to produce the desired peptide. Exceptions to this cycle program are amino acid couplings predicted to be difficult by nature of their hydrophobicity or predicted inclusion within a helical formation during synthesis. For these situations, the above cycle can be modified by repeating step 4 a second time immediately upon completion of the first 45 minute coupling step to "double couple" the amino acid of interest. Additionally, in the first coupling step in peptide synthesis, the resin can be allowed to swell for more efficient coupling by increasing the time of mixing in the initial DMF washes to three 15 minute washes rather than three 30 second washes. After peptide synthesis, the peptide can be cleaved from the resin as follows: (1) washing the peptide-resin three times for 30 seconds with DMF; (2) removing the Fmoc protective group on the amino terminus by washing two times for 15 minutes in 20% piperdine in DMF; (3) washing the peptide-resin three times for 30 seconds with DMF; and (4) mixing a cleavage cocktail consisting of 95% trifluoroacetic acid (TFA), 2.4% water, 2.4% phenol, and 0.2% triisopropysilane with the peptide-resin for two hours, then filtering the peptide in the cleavage cocktail away from the resin, and precipitating the peptide out of solution by addition of two volumes of ethyl

ether. To isolate the peptide, the ether-peptide solution can be allowed to sit at -20°C for 20 minutes, then centrifuged at 6,000xG for 5 minutes to pellet the peptide, and the peptide can be washed three times with ethyl ether to remove residual cleavage cocktail ingredients. The final peptide product can be purified by reversed phase high pressure liquid chromatography (RP-HPLC) with the primary solvent consisting of 0.1% TFA and the eluting buffer consisting of 80% acetonitrile and 0.1% TFA. The purified peptide can then be lyophilized to a powder.

In a preferred embodiment, the invention provides a peptide with branched amino acids (branched peptide), preferably a branched peptide of amino acids 45-57 (SEQ ID NO:6) with branches occurring at positions 47 and 51, respectively, instead of the Gly and Ala residues normally present. Most preferably, diaminobutyric acid is substituted for the gly and ala residues at positions 47 and 51, respectively, and proline bonded to both diaminobutyric acid residues (45-57 branched) as shown in Figure 4A.

In other specific embodiments, branched versions of the β-hCG peptides listed hereinabove are provided, *e.g.*, by substituting one or more amino acids within the β-hCG sequence with an amino acid or amino acid analog with a free side chain capable of forming a peptide bond with one or more amino acids (and thus capable of forming a "branch").

Branched peptides may be prepared by any method known in the art for covalently linking any naturally occurring or synthetic amino acid to any naturally occurring or synthetic amino acid in a peptide chain which has a side chain group able to react with the amino or carboxyl group on the amino acids so as to become covalently attached to the peptide chain. In particular, amino acids with a free amino side chain group, such as, but not limited to, diaminobutyric acid, lysine, arginine, ornithine, diaminopropionic acid and citrulline, can be incorporated into a peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free amino side group, from

that residue. Alternatively, amino acids with a free carboxyl side chain group, such as, but not limited to, glutamic acid, aspartic acid and homocitrulline, can be incorporated into the peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free carboxyl side group, from that residue. The amino acid forming the branch can be linked to a side chain group of an amino acid in the peptide chain by any type of covalent bond, including, but not limited to, peptide bonds, ester bonds and disulfide bonds. In a specific embodiment, amino acids, such as those described above, that are capable of forming a branch point, are substituted for β-hCG residues within a peptide having a β-hCG sequence.

Branched peptides can be prepared by any method known in the art. For example, but not by way of limitation, branched peptides can be prepared as follows: (1) the amino acid to be branched from the main peptide chain can be purchased as an N-α-tert-butyloxycarbonyl (Boc) protected amino acid pentafluorophenyl (Opfp) ester and the residue within the main chain to which this branched amino acid will be attached can be an N-Fmoc-α-γ-diaminobutyric acid; (2) the coupling of the Boc protected amino acid to diaminobutyric acid can be achieved by adding 5 grams of each precursor to a flask containing 150 ml DMF, along with 2.25 ml pyridine and 50 mg dimethylaminopyridine and allowing the solution to mix for 24 hours; (3) the peptide can then be extracted from the 150 ml coupling reaction by mixing the reaction with 400 ml dichlormethane (DCM) and 200 ml 0.12N HCl in a 1 liter separatory funnel, and allowing the phases to separate, saving the bottom aqueous layer and re-extracting the top layer two more times with 200 ml 0.12 N HCl; (4) the solution containing the peptide can be dehydrated by adding 2-5 grams magnesium sulfate, filtering out the magnesium sulfate, and evaporating the remaining solution to a volume of about 2-5 ml; (5) the dipeptide can then be precipitated by addition of ethyl acetate and then 2 volumes of hexanes and then collected by filtration and washed two times with cold

hexanes; and (6) the resulting filtrate can be lyophilized to achieve a light powder form of the desired dipeptide. Branched peptides prepared by this method will have a substitution of diaminobutyric acid at the amino acid position which is branched. Branched peptides containing an amino acid or amino acid analog substitution other than diaminobutyric acid can be prepared analogously to the procedure described above, using the N-F-moc coupled form of the amino acid or amino acid analog.

In a preferred embodiment, the peptide is a cyclic peptide, preferably a cyclic peptide of β-hCG amino acids 44-57 (SEQ ID NO:26) with cysteine substituted for valine at position 44 and circularized via a disulfide bond between the cysteine residues at positions 44 and 57 (C[V44C] 45-57) (Figure 4B), or a cyclic fused peptide of β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and circularized via a disulfide bond between the cysteine residues at positions 110 and 57. In another preferred embodiment, the peptide is a cyclic branched peptide of β-hCG amino acids 44-57 (SEQ ID NO:12) with cysteine substituted for valine at position 44 and circularized via a disulfide bond between the cysteine residues at positions 44 and 57 and positions 47 and 51 substituted with a diaminobutyric acid residue on which a proline is peptide bonded to its free amino sidechain.

Cyclization can be, for example, but not by way of limitation, via a disulfide bond between two cysteine residues or via an amide linkage. For example, but not by way of limitation, disulfide bridge formation can be achieved by (1) dissolving the purified peptide at a concentration of between 0.1.-0.5 mg/ml in 0.01 M ammonium acetate, pH 7.5; (2) adding to the dissolved peptide 0.01 M potassium ferricyanide dropwise until the solution appears pale yellow in color and allowing this solution to mix for 24 hours; and (3) concentrating the cyclized peptide to 5-10 ml of solution, repurifying the peptide by reverse phase-high

pressure liquid chromatography (RP-HPLC) and finally lyophilizing the peptide. In a specific embodiment, in which the peptide does not contain two appropriately situated cysteine residues, cysteine residues can be introduced at the amino-terminus and/or carboxy-terminus and/or internally such that the peptide to be cyclized contains two cysteine residues spaced such that the residues can form a disulfide bridge. Alternatively, a cyclic peptide formed by an amide linkage can be obtained by, for example but not limited to, the following procedure: An allyl protected amino acid, such as aspartate, glutamate, asparagine or glutamine, can be incorporated into the peptide as the first amino acid, and then the remaining amino acids are coupled on. The allyl protective group can be removed by a two hour mixing of the peptide-resin with a solution of tetrakistriphenylphophine palladium (0) in a solution of chloroform containing 5% acetic acid and 2.5% N-methylmorpholine. The peptide resin can be washed three times with 0.5% N,N-diisopropylethylamine (DIEA) and 0.5% sodium diethyldithiocabamate in DMF. The amino terminal Fmoc group on the peptide chain can be removed by two incubations for 15 minutes each in 20% piperdine in DMF, and washed three times with DMF for 30 seconds each. The activator mix, N-methylmorpholine and HBTU in DMF, can be brought onto the column and allowed to couple the free amino terminal end to the carboxyl group generated by removal of the allyl group to cyclize the peptide. The peptide can cleaved from the resin as described in the general description of chemical peptide synthesis above and the peptide purified by reverse phase-high pressure liquid chromatography (RP-HPLC). In a specific embodiment, in which the peptide to be cyclized does not contain an allyl protected amino acid, an allyl protected amino acid can be introduced into the sequence of the peptide, at the amino-terminus, carboxy-terminus or internally, such that the peptide can be cyclized.

β-hCG peptides can also be obtained by recombinant expression techniques. (See, *e.g.*, Sambrook et al., 1989,

Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 2d Ed., Cold Spring Harbor, New York, Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K., Vol. I, II). The nucleic acid sequence encoding hCG has been cloned and the sequence determined (see Figure 3 and Xia, H., 1993, *J. Molecular Endocrinology* June 10; 1993:337-343; Sherman, G.B., 1992, *J. Molecular Endocrinology*, June 6, 1992:951-959; Gieseman, L.K. (ed.), 1991, *Basic and Chemical Endocrinology*, pp. 543-567; Ward et al., 1991, in *Reproduction in Domestic Animals*, 4th ed., P.T. Coppos, ed., pp. 25-80, Academic Press, New York) and can be isolated using well-known techniques in the art, such as screening a library, chemical synthesis, or polymerase chain reaction (PCR).

To recombinantly produce β-hCG peptides, nucleic acid sequence encoding the β-hCG peptide is operatively linked to a promoter such that the β-hCG peptide is produced from said sequence. For example, a vector can be introduced into a cell, within which cell the vector or a portion thereof is expressed, producing the β-hCG peptide. In a preferred embodiment, the nucleic acid is DNA if the source of RNA polymerase is DNA-directed RNA polymerase, but the nucleic acid may also be RNA if the source of polymerase is RNA-directed RNA polymerase or if reverse transcriptase is present in the cell or provided to produce DNA from the RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in bacterial or mammalian cells. Expression of the sequence encoding the β-hCG peptide can be by any promoter known in the art to act in bacterial or mammalian cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3'

long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell* 22:787-797), the HSV-1 (herpes simplex virus-1) thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42), etc., as well as the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Ornitz et al., 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer et al., 1987, *Science* 235:53-58), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, *Genes and Devel.* 1:161-171), beta-globin gene control region which is active in erythroid cells (Mogram et al., 1985, *Nature* 315:338-340; Kollias et al., 1986, *Cell* 46, 89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-286), and gonadotropin releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, *Science* 234:1372-1378). The promoter element which is operatively linked to the nucleic acid encoding the β-hCG peptide can also be a

bacteriophage promoter with the source of the bacteriophage RNA polymerase expressed from a gene for the RNA polymerase on a separate plasmid, *e.g.*, under the control of an inducible promoter, for example, a nucleic acid encoding the β-hCG peptide operatively linked to the T7 RNA polymerase promoter with a separate plasmid encoding the T7 RNA polymerase.

In a less preferred embodiment, peptides can be obtained by proteolysis of hCG followed by purification using standard techniques such as chromatography (*e.g.*, HPLC), electrophoresis, etc.

Also included within the scope of the invention are β-hCG peptide derivatives which are differentially modified during or after synthesis, *e.g.*, by benzylation, glycosylation, acetylation, phosphorylation, amidation, pegylation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. In specific embodiments, the peptides are acetylated at the N-terminus and/or amidated at the C-terminus. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In another embodiment, the β-hCG peptide derivative is a chimeric, or fusion, protein comprising a functional β-hCG peptide joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a β-hCG-coding sequence joined in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a

chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer.

6.1.3. FRACTIONS OF SOURCES OF hCG OR β-hCG

In another embodiment, a fraction, particularly a size fraction, of a source of native hCG or native β-hCG (*i.e.* naturally occurring, not recombinantly produced, hCG or β-hCG) active in inhibiting cancer cell growth, particularly a size fraction of approximately 40 kD, 15 kD or 2-3 kD, is used to treat or prevent cancer. The utility of fractions of native hCG and native β-hCG sources may be determined by the *in vitro* and *in vivo* assays described in Section 6.2 *infra* or by any other method known in the art.

The present inventors have found that different preparations of native hCG and native β-hCG have variable effects on HIV infection both *in vitro* and *in vivo*. Specifically, the inventors found that among the commercial preparations of (non-recombinant) hCG they investigated, hCG from Fujisawa was the most effective, hCG APL™ (Wyeth-Ayerst) the next most effective, and PREGNYL™ (Organon) the next most effective in inhibiting HIV infection and replication and Kaposi's Sarcoma. A highly purified hCG preparation and recombinant β-hCG were found not to be active in inhibiting HIV infection and Kaposi's Sarcoma cell growth *in vitro*. In fact, the present inventors have shown that specific size fractions of an active hCG preparation (APL™; Wyeth Ayerst) and of human early (*i.e.* first trimester) pregnancy urine have anti-HIV activity in vitro and anti-KS activity both in vitro and in vivo, as described *infra* in Section 8. These active fractions were eluted from the sizing matrix as or close to (i.e., within 5 fractions (where the fractions are 4 ml fractions using the SUPERDEX™ 200 column which is 26 mm^2 by 60 mm)) the fractions containing or that would contain material that is approximately 40 kD (±8 kD), 15 kD (±3 kD) and 2-3 kD (±2 kD) molecular weight. One skilled in the art would understand that these fractions could be subjected to further size fractionation to further isolate the component

of these fractions having the anti-HIV and/or anti-KS activity. Additionally, other methods of fractionation, such as ion-exchange chromatography, affinity chromatography are well known in the art; those skilled in the art would be able to use any available fractionation techniques to obtain the active fractions from the active hCG preparations and human early (first trimester) pregnancy urine. hCG preparations and fractions of hCG preparations can be screened for efficacy in treating or preventing HIV infection by the assays described in Sections 6.2, 7 and 8 *infra* or by any method known in the art.

In a specific embodiment, the invention provides a first composition comprising one or more first components of a second composition comprising native hCG or native β-hCG, said first components being separated from other components of the hCG or β-hCG sample, said first components being active to inhibit cancer cell growth, and said second composition being active to inhibit cancer cell growth, and said native hCG or native β-hCG not being purified to homogeneity in said second composition. In particular the invention provides a composition comprising components which have been separated from other components of the native hCG or native β-hCG sample by sizing column chromatography, preferably where the components elute from a gel filtration, preferably a SUPERDEX™ 200, sizing column with an apparent approximate molecular weight of 40 kD, 14 kD or 2-3 kD as determined relative to the elution of a hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein (β-hCG amino acids 6-40 linked via a disulfide bond to β-hCG amino acids 55-92, as depicted in Figure 3 (SEQ ID NO:2)), having a molecular weight of 10 kD.

6.1.4. SOURCES OF hCG AND β-hCG

Native preparations (*i.e.* derived from naturally occurring sources and not recombinantly produced) of hCG and β-hCG can be obtained from a variety of sources. Both hCG and β-hCG are commercially available (*e.g.*, Sigma Chemical

Company) and hCG is commercially available in a form suitable for therapeutic use in humans (*e.g.*, from Fujisawa, Wyeth-Ayerst Laboratories (APL™), Organon, Inc. (PREGNYL™) and Serono Laboratories, Inc. (PROFASI™)). hCG is also present at particularly high concentrations in the urine of women in the first trimester of pregnancy ("human early pregnancy urine"). Other sources include, but are not limited to, urine from women in the second and third trimesters of pregnancy, urine from patients with proteinuria, urine from patients having hCG secreting tumors or other cancer patients, and from pituitary glands.

Since the inventors have discovered that different sources of hCG have variable effects on HIV infection and cancer cell growth *in vitro* and *in vivo*, one aspect of the invention relates to assaying preparations of hCG for efficacy in treatment or prevention of cancer. The therapeutic effectiveness of hCG preparations and fractions can be tested by the *in vitro* or *in vivo* assays described in Section 6.2 *infra* or by any method known in the art. It is preferable to test the hCG preparation or fraction in an *in vitro* assay, *e.g.*, for inhibition of cancer cell growth or *in vivo* in an animal model, such as KS tumors induced in mice, before assaying the preparation in humans.

In a specific embodiment, a preparation comprising hCG is used that contains not only the hCG heterodimer but also peptide fragments thereof, *e.g.*, β chain peptides.

hCG and β-hCG can also be purified, or preferably partially purified, from any source known to contain hCG or β-hCG, *e.g.*, urine from pregnant women, using conventional techniques well-known in the art, such as affinity chromatography. For example, antibodies prepared against hCG or β-hCG can be used to prepare an affinity chromatography column which can be used to purify the proteins by well-known techniques (*see, e.g.*, Hudson & May, 1986, *Practical Immunology*, Blackwell Scientific Publications, Oxford, United Kingdom).

The β-hCG-related proteins are preferably prepared by any chemical or enzymatic synthesis method known in the art, as described *supra* in Section 6.1.2.

6.1.5. FRACTIONATION OF SOURCES OF hCG

The present inventors have found that the component(s) of a source of hCG having anti-HIV and/or anti-KS (or other anti-cancer) activity can be further isolated by fractionation of the source of hCG. The inventors have fractionated the active portions of the commercial hCG preparation APL™ (Wyeth-Ayerst) and human early pregnancy urine as described in Section 8 *infra*. Other sources of hCG include, but are not limited to, urine from women in the second and third trimester of pregnancy, urine from proteinuria patients (both pregnant women with preeclampsia and patients with nephrotic syndromes), urine from patients with hCG secreting tumors, and pituitary glands. However, those skilled in the art will appreciate that any source of hCG or β-hCG having anti-HIV activity and/or anti-KS activity and/or a pro-hematopoietic effect can be fractionated to further isolate the active components. The source of hCG or β-hCG can be fractionated using any technique available in the art for the separation and isolation of molecules, for example but not limited to, sizing chromatography, ion-exchange chromatography, affinity chromatography, etc.

Briefly, by way of example but not by way of limitation, urine can be prepared for fractionation as follows:

Urine is collected and stored either frozen or refrigerated for not more than two (2) days. Then, sodium azide is then added at a concentration of 1 gram/liter and the sample is stored frozen until sufficient material is collected for the fractionation.

At this point, the urine is thawed over night, the pH adjusted to 7.2 to 7.4 with sodium hydroxide and then centrifuged to remove any precipitate (alternatively, the precipitate can be allowed to sediment, *e.g.*, for 1 hour at room temperature, approximately 75% of the

supernatant is decanted, the remainder of the supernatant and the precipitate is centrifuged to pellet the precipitate, and the supernatant decanted and added to the first volume of decanted supernatant). The urine is then filtered through, *e.g.*, a 45 micron filter to remove any remaining particulate matter.

Next, the urine is concentrated using any concentration method available in the art which does not remove higher molecular weight material, *e.g.*, material larger than 3,000 daltons in molecular weight; for example, the material may be concentrated using a Pellicon (Millipore) filtration system with a membrane filter cassette having a molecular weight cut off of 3,000 daltons. Concentration with the Pellicon filtration system using the 3,000 molecular weight membrane filter cut off concentrates 30 liters of urine to 500 ml (i.e., a 60-fold concentration) overnight.

To remove salts and lipids, the concentrate can then be passed over a column containing a large volume of Sephadex G25 resin in 0.05 M ammonium bicarbonate (for example, 250 ml of the concentrate can be passed over a column of approximately 1.7 liters, washing the column with 25% ethanol between runs to remove adsorbed lipids and glycoprotein). The resulting desalted and delipidated urine concentrate is then lyophilized.

The lyophilized urine material or commercial hCG preparation (or any source of native hCG or β-hCG) is resuspended in either phosphate buffered saline (PBS-- 30 mM sodium phosphate buffer, pH 8.3) or in 0.10 M ammonium bicarbonate at a concentration and in a volume appropriate for the column upon which the sample will be loaded, for example, but not limited to 0.5 grams of protein in 6 ml (i.e., approximately 83 mg/ml). It is within the skill of the skilled artisan to determine the concentration and volume of the sample to be subjected to fractionation.

The sample can then be fractionated by any method known in the art for the separation of proteins. A preferred

method is high resolution gel filtration on a Pharmacia pre-packed SUPERDEX™ 200 column (26/60) by HPLC using any available HPLC apparatus, *e.g.*, with a Hewlett Packard 1050 HPLC equipped with a photodiode array detector. The resuspended sample is loaded onto the column in 30 mM phosphate buffer, pH 8.3, and the material can then be eluted from the column with 30 mM sodium phosphate buffer, pH 7.0; 2M NaCl in, *e.g.* 4 ml fractions. Fractionation can also be performed using other types of chromatography matrices e.g., heparin, DEAE-cellulose, Sephadex A50, Sephadex G100, phenyl sepharose, or any sizing, ion-exchange, affinity chromatography or any other chromatography matrix available in the art. The column chromatography can also be run using any method available in the art, e.g., standard gravity flow or low pressure chromatography, FPLC, or reverse phase HPLC.

Many separation techniques are known in the art. Those skilled in the art would understand how to apply these known techniques to the fractionation of hCG preparations.

Once the material has been fractionated, any method known in the art, such as but not limited to, those described in sections 6.2, 7, and 8 *infra*, can be used to determine which fractions have anti-HIV activity and/or anti-KS (or other anti-cancer) activity and/or a pro-hematopoietic effect.

When fractionating by size, such as fractionation on the SUPERDEX™ 200 column, the apparent molecular weight of material in the fractions can be determined by the relative elution of those fractions compared with the elution of specific hCG and β-hCG species having a known molecular weight or with the elution of known protein size markers. In general, proteins elute from a sizing column as a function of their molecular weight. The elution of, for example, hCG and the β-hCG core protein can be determined by assaying the column chromatography fractions for the presence of hCG and the β-hCG core protein, or any hCG or β-hCG species, by any immunoassay technique available in the art, such as

radioimmunoassays (either liquid or solid phase), enzyme-linked assays or ELISA assays.

Antibodies, either polyclonal or, preferably, monoclonal, can be generated against hCG or the β-hCG core protein by any method known in the art. Preparation of monoclonal antibodies against hCG and β-hCG species have been described in the art, *see, e.g.*, O'Connor et al., 1994, *Endocrine Reviews* 15:650-683; Krichevsky et al, 1991, *Endocrinology* 128:1255-1264; and Krichevsky et al., 1988, *Endorcrinology* 123:584-593. For the production of antibodies, various host animals can be immunized by injection with hCG, the β-hCG core protein or any other species of hCG, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. For preparation of monoclonal antibodies, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Monoclonal cells lines can then be screened for binding to the particular hCG or β-hCG species using the purified species in any type of immunoassay available in the art (*see, e.g.*, Erlich et al., 1985, *Am. J. Reprod Immunol. Microbiol.* 8:48).

The fractions can then be assayed for the presence of the hCG or β-hCG species using a monoclonal antibody specific

for the hCG or β-hCG species. The assay can be performed by any method known in the art. For example, an immunoradiometric assay (IRMA) can be used (Krichevsky et al., 1988, *Endocrinology* 123:584-593). Briefly, the IRMA assay is performed by adsorbing an antibody against the hCG or β-hCG species onto the surface of wells of a microtiter plate by incubation in a coating buffer (0.2 M sodium bicarbonate, pH 9.5) overnight at 4°C. The residual non-specific binding sites are blocked by the addition of a 1% bovine serum albumin solution (with 0.1% sodium azide) to the wells for 3 hours at room temperature, and the wells of the microtiter plate are then washed with deionized water. An aliquot of the fraction in assay buffer (0.01 M sodium phosphate, 0.15 M NaCl, 0.01 M EDTA, 0.1% sodium azide, 0.1% bovine γ-globulin, pH 7.4) is incubated in the wells for 24 hours at room temperature. The sample is then removed and the wells washed with deionized water. A solution of a second antibody specific for the hCG or β-hCG species, which antibody has been iodinated with I^{125}, (approximately 40,000 cpm/well) is incubated in the wells for 24 hours at room temperature. The iodinated antibody solution is removed and the wells washed five times with deionized water. The level of radioactivity in each well is then determined in a scintillation counter which can measure γ-irradiation.

6.1.6. GENE THERAPY

In a specific embodiment, nucleic acids comprising a sequence encoding β-hCG, a β-hCG peptide, or fused β-hCG peptides (*i.e.*, two or more β-hCG peptides linked at the N-termini and C-termini via peptide bond(s)), are administered for treatment or prevention of cancer, by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. In this embodiment of the invention, the nucleic acid produces its encoded protein that mediates a therapeutic effect by preventing or treating cancer. In a preferred embodiment, β-hCG or a β-hCG peptide are provided to treat or prevent

Kaposi's sarcoma, or a carcinoma of the breast, prostate, lung or kidney (renal). For example, any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIBTECH* 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

In a preferred aspect, a nucleic acid encoding β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion protein is part of an expression vector that produces β-hCG or the β-hCG peptide or fused β-hCG peptides or related fusion protein in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the nucleic acid sequence coding for β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion protein, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which the β-hCG sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the hCG nucleic acid (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then administered to the patient.

These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the cell or nucleus, *e.g.*, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In a specific embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO92/06180 dated April 16, 1992 (Wu et al.); WO92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Clarke et al.), WO93/20221 dated October 14, 1993 (Young)). In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

In a specific embodiment, a viral vector that contains the nucleic acid sequence encoding β-hCG or a β-hCG peptide

or fused β-hCG peptides or related fusion protein is used. For example, a retroviral vector can be used (see Miller et al., 1993, *Meth. Enzymol.* 217:581-599). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome. Retroviral vectors are maintained in infected cells by integration into genomic sites upon cell division. The nucleic acid to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., 1994, *Biotherapy* 6:291-302, which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, *J. Clin. Invest.* 93:644-651; Kiem et al., 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; and Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300.) Herpes viruses are other viruses that can also be used.

Another approach to gene therapy, involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including, but not limited to, transfection, electroporation, microinjection, infection with a viral vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see *e.g.*, Loeffler and Behr, 1993, *Meth. Enzymol.* 217:599-618; Cohen et al., 1993, *Meth. Enzymol.* 217:618-644; Cline, 1985, *Pharmac. Ther.* 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, epithelial cells are injected, *e.g.*, subcutaneously. In another embodiment, recombinant skin cells (*e.g.*, keratinocytes) may be applied as a skin graft onto the patient. Recombinant blood cells (*e.g.*,

hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

In an embodiment in which recombinant cells are used in gene therapy, a nucleic acid sequence coding for β-hCG or a β-hCG peptide or fused β-hCG peptide or related fusion peptide is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention.

6.2. DEMONSTRATION OF THERAPEUTIC UTILITY

The Therapeutics of the invention are preferably tested *in vitro*, and then *in vivo*, for the desired therapeutic or prophylactic activity, prior to use in humans.

One embodiment provides a method for screening a preparation comprising a protein, preferably a purified protein, having a sequence of one or more portions of β-hCG or a derivative, preferably in purified form, of said protein, or a fraction of a source of native hCG or native β-hCG for anti-cancer activity comprising assaying said preparation for the ability to inhibit the survival or proliferation of malignant cells. In a specific embodiment, the preparation is screened by a method comprising measuring the survival or proliferation of malignant cells, which cells have been contacted with the preparation; and comparing the survival or proliferation of the cells contacted with the preparation with the survival or proliferation of cells not so contacted with the preparation, wherein a lower level of survival or proliferation in said contacted cells indicates that the preparation has anti-cancer activity. In another specific embodiment, the preparation is screened by a method

comprising measuring the survival or proliferation of cells from a cell line which is derived from or displays characteristics associated with a malignant disorder, which cells have been contacted with the preparation; and comparing the survival or proliferation in the cells which have been contacted with the preparation with said survival or proliferation in cells not so contacted, wherein a lower level in said contacted cells indicates that the preparation has anti-tumor activity.

Another embodiment provides a method for screening a preparation comprising a protein having a sequence of a portion of β-hCG or a derivative of said protein or a fraction of a source of native hCG or native β-hCG, for anti-cancer activity comprising assaying said preparation for the ability to convert cells having an abnormal phenotype to a more normal cell phenotype. In a specific embodiment, the preparation is screened by a method comprising assessing the phenotype of cells suspected of being pre-neoplastic in culture, which cells have been contacted with the preparation; and comparing the phenotype in the cells which have been contacted with the preparation with said phenotype in cells not so contacted, wherein a more normal phenotype in said contacted cells indicates that the preparation has anti-cancer activity. In another specific embodiment, the preparation is screened by a method comprising assessing the phenotype of cells from a cell line which is derived from or displays characteristics associated with a pre-malignant disorder, which cells have been contacted with the preparation; and comparing the phenotype in the cells which have been contacted with the preparation with said phenotype in cells not so contacted, wherein a more normal phenotype in said contacted cells indicates that the preparation has anti-cancer activity.

Yet another embodiment provides a method for screening a preparation comprising a protein having a sequence of a portion of β-hCG or a derivative of said protein or a fraction of a source of native hCG or native β-hCG, for

activity in treatment or prevention of Kaposi's Sarcoma comprising assaying said preparation for the ability to inhibit Kaposi's Sarcoma cell proliferation or promote Kaposi's Sarcoma cell apoptosis. In a specific embodiment, the preparation is screened by a method comprising measuring proliferation or colony formation in cultured KS Y-1 or KS-SLK cells, which cells have been contacted with the preparation; and comparing the measured proliferation or colony formation in the cells which have been contacted with the preparation with said proliferation or colony formation in cells not so contacted with the preparation, wherein a lower level of proliferation or colony formation in said contacted cells indicates that the preparation has anti-Kaposi's Sarcoma activity.

In another specific embodiment, the preparation is screened by a method comprising measuring apoptosis in a Kaposi's Sarcoma tumor in an immunodeficient mouse, which Kaposi's Sarcoma tumors have been induced by injection with KS Y-1 or KS-SLK cells, and which mouse has been exposed to the preparation; and comparing the degree of apoptosis in the tumor of the mouse which has been exposed to the preparation with a tumor in a mouse not so exposed, wherein a higher in level of apoptosis in the tumor of said exposed mouse indicates that the preparation has anti-Kaposi's Sarcoma activity.

For example, *in vitro* assays which can be used to determine whether administration of a specific Therapeutic is indicated include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a Therapeutic, and the effect of such Therapeutic upon the tissue sample is observed. In one embodiment, where the patient has a malignancy, a sample of cells from such malignancy is plated out or grown in culture, and the cells are then exposed to a Therapeutic. A Therapeutic which inhibits survival or growth of the malignant cells is selected for therapeutic use *in vivo*. Many assays standard in the art can be used to assess such

survival and/or growth; for example, cell proliferation can be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g., fos, myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc.

In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a Therapeutic has a desired effect upon such cell types.

In another embodiment, cells of a patient tissue sample suspected of being pre-neoplastic are similarly plated out or grown *in vitro*, and exposed to a Therapeutic. The Therapeutic which results in a cell phenotype that is more normal (*i.e.*, less representative of a pre-neoplastic state, neoplastic state, malignant state, or transformed phenotype) is selected for therapeutic use. Many assays standard in the art can be used to assess whether a pre-neoplastic state, neoplastic state, or a transformed or malignant phenotype, is present. For example, characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*) include a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton surface protein, etc. (see Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York pp. 436-446).

In other specific embodiments, the *in vitro* assays described *supra* can be carried out using a cell line, rather than a cell sample derived from the specific patient to be treated, in which the cell line is derived from or displays characteristic(s) associated with the malignant, neoplastic or pre-neoplastic disorder desired to be treated or

prevented, or is derived from the cell type upon which an effect is desired, according to the present invention.

Specifically, Therapeutics can be tested for efficacy in treatment or prevention of Kaposi's sarcoma by any of the methods relating to Kaposi's sarcoma described in Section 6 *infra* or in Lunardi-Iskandar et al. (1995, Nature 375:64-68) or by any other method known in the art. Briefly, KS cell lines, KS Y-1 (*Ibid.*) or KS-SLK (Siegal, B. et al., 1990, *Cancer* 65:492-498), which will produce malignant tumors in immunodeficient mice, are used to perform *in vitro* proliferation and clonogenic assays (*see, e.g.*, Lunardi-Iskandar, Y. et al., 1993,*J. Exp. Med.* 177:741-750); methods for performing such assays are well known in the art. A Therapeutic which reduces proliferation or colony formation in the cultured cells can be used in the methods of the invention for treatment or prevention of KS.

Efficacy of a Therapeutic can also be determined by administration of the Therapeutic to immunodeficient mice injected with either the KS-Y-1 or KS-SLK cells, which cause tumor formation in the mice, and assessment of the degree of apoptosis and angiogenesis of tumor cells after treatment with the Therapeutic. Apoptosis is detected by staining fixed tissue samples from the tumor for the presence of cells with DNA fragmentation. For example, this is accomplished by treating tissue slides from formalin-fixed tumors with terminal deoxynucleotide transferase for extension of DNA ends (3' hydroxyl ends) and incorporation of digoxigenin-11-dUTP. Anti-digoxigenin antibody conjugated with the enzyme peroxidase allows detection of apoptotic cells that stain brown whereas viable cells stain blue. An increase in KS tumor cell apoptosis and a decrease in angiogenesis indicates that the Therapeutic has utility in treatment of KS.

The Therapeutic can also be assessed in clinical trials in human patients suffering from KS or any other cancer. To test the efficacy of the Therapeutic in KS patients, either local, *i.e.* intralesional, or systemic administration of the Therapeutic can be used. Tumors can be examined physically

for regression in response to administration of the Therapeutic. Additionally, tissue biopsies can be taken from the tumors, and these tissue samples examined for apoptosis, as described above.

Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used.

6.3. THERAPEUTIC COMPOSITIONS AND METHODS OF ADMINISTRATION

The invention provides methods of treatment and prevention by administration to a subject of an effective amount of a Therapeutic of the invention. The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In a specific embodiment, the subject is a human not afflicted with a cancer which secretes hCG or hCG fragments and, more particularly, not afflicted with Kaposi's Sarcoma.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the Therapeutic, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may

be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example and not by way of limitation, by topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In a preferred embodiment, the pharmaceutical composition of the invention is injected into a KS lesion.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (*see* Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; *see* generally *ibid.*)

In yet another embodiment, the Therapeutic can be delivered in a controlled release system. In one embodiment, a pump may be used (*see* Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); *see also* Levy et al., *Science* 228:190 (1985); During et al.,

Ann. Neurol. 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the systemic dose (*see, e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered by gene therapy methods as described *supra* Section 6.1.4.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying

agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those

derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vivo* and/or *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. For treatment of KS, suitable dosages include, but are not limited to, 1,000 to 5,000 I.U. hCG for intralesional injection up to seven days per week and/or 20,000 I.U. intramuscularly (or intravenously or subcutaneously) two times per week in human patients. Doses up to 45,000 I.U. per week were also well tolerated by human patients. Predicted suitable doses of β-hCG peptide administered intralesionally include, but are not limited to, 0.1 to 10 micrograms up to and including seven days per week for human patients. For systemic administration, for example but not limited to, intramuscularly, intravenously or subcutaneously, in a specific embodiment, weekly doses of 1 to 1000 micrograms of β-hCG peptide are predicted to be suitable for a human patient. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of

the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

7. EXAMPLE: EFFECTS OF hCG AND β-hCG PREPARATIONS AND β-hCG PEPTIDES ON KAPOSI'S SARCOMA

As described herein, we have observed beneficial effects of some preparations of human Chorionic Gonadotropin (hCG) against HIV disease including anti-tumor (Kaposi sarcoma, KS), anti-viral, increase in weight and pro-hematopoiesis effects. Our studies document that the same preparations inhibit KS cell growth *in vitro* and induce apoptosis in a mouse model. Examples of these effects were also noted in some HIV-positive patients treated with some hCG preparations. The strength of these effects varied among crude hCG preparations, and highly purified hCG did not retain these activities. However, the anti-KS, anti-viral, and pro-hematopoietic effects were mimicked by native β-hCG and synthetic peptides of the beta subunit of hCG having amino acid sequences of amino acid numbers 45-57 (SEQ ID NO:6), 109-119 (SEQ ID NO:7), circularized 44-57, where cysteine is substituted for the amino acid at position 44 (SEQ ID NO:26), and peptides of amino acid numbers 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or a peptide of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 3 (portions of SEQ ID NO:2). The peptides having an amino acid sequence of amino acid numbers 7-45, 47-55, 46-65, and 48-56 (SEQ ID NOS:21 and 33-35, respectively) of β-hCG (Figure 3 (SEQ ID NO:2) also exhibit activity in *in vitro* assays.

7.1. EARLY STUDIES OF SOME hCG PREPARATIONS IN PATIENTS WITH HIV-1 DISEASE

The incidence of KS is greatly increased in HIV-infected persons (Friedman-Kien et al., 1981, *J. Am. Acad. Dermatol.* 5:468-473). Based on experimental studies of the killing effect of some hCG preparations on KS Y-1 cells, clinical trials with some commercially available preparations of hCG given either intralesionally (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269, Harris, P.J., 1995, *The Lancet* 346:118-119) or systemically to KS patients have shown that cutaneous KS lesions were reduced via cell killing by apoptosis following intralesional inoculation (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med* 335: 1261-1269) and induced regression of advanced KS disease treated by systemic delivery.

Clinical trials reported herein were undertaken in Belgium and California to evaluate the anti-KS properties of systemic hCG therapy with or with out concomitant intralesional therapy. Use of anti-viral protease and non-protease inhibitors was not restricted. A total of 47 patients were enrolled under protocols of compassionate use sanctioned by the Institutional Review Boards of the respective centers. 29 patients were treated in Belgium, either on a protocol to investigate intralesional and systemic treatment of cutaneous KS (n=15), or in the pre-clinical phase of that protocol (n=4), or on compassionate use for systemic KS or HIV infection (n=10). The protocol involved intralesional administration of 500 IU hCG (PREGNYL™) to 4 lesions for 2 weeks, followed by subcutaneous administration of 2,500 IU hCG (PREGNYL™) 5 days per week for 4 to 6 weeks. Additional systemic intramuscular or subcutaneous hCG treatment with either PREGNYL™, APL™, or STERIS™ (one patient) was provided as ongoing therapy in some patients or as part of compassionate use protocols.

A total of 18 patients were treated in California with at least 1 month of follow-up as part of an ongoing protocol to evaluate systemic hCG therapy for cutaneous KS. These patients received either 5000 IU of APL™ subcutaneously 7 days per week, 10,000 IU subcutaneously 3 times per week, or 10,000 IU subcutaneously 7 days per week.

Overall 30 patients were on pre-existing, anti-viral therapy (19 on RT inhibitors and 11 on protease inhibitors), 11 were on no anti-virals and 8 were missing information. One patient, PH-RF, was on 3TC therapy before hCG therapy, and despite poor compliance, had an hCG response for visceral KS and viral load, which declined to undetectable on hCG alone.

Thirty-six patients survived the study, 7 (PH-LFA, PH-DD, PH-PJ, PO-BO, PO-RB, PH-JJ, PH-MH) died either from opportunistic infections or multiple organ failure. The vital status of 1 patient is unknown. Two patients, PH-DD and PH-OJ, discontinued hCG treatment because of cholestasis. PH-DD was on concomitant anti-mycobacterial therapy which was felt to be a contributing factor. PH-OJ had preexisting cholestasis, which was exacerbated by the hCG treatment with a marked increase in alkaline phosphatase and rise in bilirubin which required hospitalization (PH). These values declined by 2-fold following discontinuation of hCG therapy. These cases raise the possibility that liver toxicity may be a rare complication of hCG therapy.

Early clinical experience with relatively low dose intralesional hCG administration for KS indicated partial or complete regression of treated lesions, including 3 of the first 4 patients in the initial pilot study in Belgium (Hermans et al.,1995, *Cellular and Molecular Biology* 3:357-364) as well as a dose dependent effect between 16% (250 IU) and 83% (2,000 IU) in patients reported from California (Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269), and other cases showing striking clearance of visceral (lung and gastrointestinal) KS in very advanced disease

following systemic therapy with hCG APL™ or PREGNYL™ within 1 to 3 months of initiating therapy.

Among the 30 cases with cutaneous Kaposi's Sarcoma, 12 were treated with intralesional followed by systemic therapy in Belgium and 18 with systemic therapy only in California. Complete (2/12, Belgium; 2/18, California) and partial (5/12, Belgium; 4/18, California) responses were observed while progressive disease was noted among 2/12 from Belgium and 10/18 in California. The overall response rate for the study (CR + PR) was 43% (13/30). The response rate in the group administered hCG both intralesionally plus systemicly group was 58%, while the response rate was 33% in the group receiving only the systemic treatment. Among 8 patients with both visceral and cutaneous KS treated in Belgium with very advanced pulmonary or gastric lesions, 3 patients experienced complete remissions, 2 patients exhibited tumor stabilization and 3 progressed, in each case after failure of conventional cytotoxic therapy.

AIDS patients treated with hCG therapy were tested for increases in $CD4^+$ T cell levels (in numbers of cells per mm^3) and decrease in viral load by one of the following assays for determining viral load: NASBA (Louache, et al., 1992, *Blood* 180:2991-2999; Geller, et al., 1985, *Archs. Path. Lab. Met.* 109:138-145), which has a lower detection limit of 4,000 copies; Roche Amplicor, with a lower detection limit of 200 copies; RT-PCR, with a lower detection limit of 100 copies; or TCID assay in which the infection of PBMCs in co-culture is determined (Popovic et al., 1984, *Science* 204:497-500). As viral load was assayed retrospectively, the viral load results were not a factor in guiding choice of therapy or changes in therapy. Each patient served as their own control and change in viral load (0.7 log change between baseline and subsequent post hCG viral load, scored as significant) was the endpoint measurement for this analysis. For analysis of the anti-viral effect, in addition to the 10 patients undergoing with synchronous hCG and other anti-viral therapy, 6 patients were excluded because of a lack of base line viral

load or insufficient follow up before hCG therapy was stopped or additional anti-viral therapy was started.

Among the 16 cases, 1 (PH-OJ) experienced a fall in viral load of 0.7 log on 2 successive tests at least 1 month apart while on stable anti-viral therapy (see Figures 1A and B), 11 were non responding and 2 (PH-VE and PHGRX) manifested an increase in viral load of at least 0.7 log after hCG therapy on 2 successive tests at least 1 month apart. As illustrated in Figures 1C and D, another patient (PG-1), initially on hCG alone and classified as non responsive by study criteria (2 consecutive values of 0.7 log decrease in viral load over 1 month) on hCG alone, experienced a steady decline in viral load but the second qualifying >0.7 log viral load drop was measured 2 weeks after non protease inhibitor therapy was begun. Because of this short window, it is likely that this second stable viral load point is accounted for by hCG rather than the newly introduced anti-virals. It is noteworthy that $CD4^+$ T cell levels were not significantly altered in this case but, the patient's KS progressed, documenting a dissociation of various hCG effects.

Among the 6 cases being treated with hCG alone (i.e. without other anti-viral therapies) with analyzable data, all were scored as non responsive to the hCG therapy by the scoring criteria although one case (PG-1) noted above (and illustrated in Figures 1C and D) is a probable responder. An additional patient on hCG alone (PG-8; Figures 1E and F) experienced a sustained fall in viral load of 0.5 log over a 2.8 month period of treatment on hCG alone until KS lesions progressed, at which time hCG therapy was discontinued. Thus of the 7 analyzable patients on hCG alone, 4 exhibited a downward trend in viral load, 2 patients showed an increase in viral load, and 1 patient was stable.

To more fully evaluate all data from patients on hCG alone or with stable antiviral therapy, all eligible data points were plotted, as shown in Figure 8A, indicating the coordinates for each data point pre and post therapy, with

values on the line representing no change in viral load. Values are distributed more or less equally above and below the line with no obvious trend to suggest a strong anti viral effect. To evaluate a dose response relationship between hCG and viral load, regression analysis for patients on hCG, alone or with stable antiviral therapy is shown in Figure 8C. There was no detectable effect of higher hCG dose on viral load level (r=-.089, p=0.285, N=147 measurements). An analysis by different CD4 strata did not show any significant trends to suggest that level of immunity impacted the hCG effect.

Among the 22 patients with analyzable $CD4^+$ T cell data, 5 demonstrated a pro-$CD4^+$ T cell effect (PH-VE, PH-RF, PG-9, PG-17, and PG-19) characterized by a 50% rise in $CD4^+$ T cell count sustained over at least a one month period, as demonstrated by plotting the data from at least two patients (PH-VE--Figures 1G and H and PG-17--Figures 1I and J). Of these 5 patients, concomitant stable non protease anti-virals were administered to 2 patients, stable protease inhibitors in 2 cases and hCG preparation alone in 1 case. Thus of the 6 cases with valid $CD4^+$ T cell data on hCG preparation alone, 1 manifested a significant response. No patient experienced an adverse fall in $CD4^+$ T cell on hCG preparation therapy, although patient PH-VE experienced an 0.7 log rise in viral load with a sustained 50% fall in $CD4^+$ T cell numbers and a partial anti KS response (Figures 1G and H). Similarly, paient PG-17 experienced a significant rise in $CD4^+$ T cells and no change in viral load on hCG therapy alone, yet experienced progression of KS after 2.5 months (Figures 1I and J). All $CD4^+$ T cell values (except for 2 patients on hCG alone) were at or above baseline, with the most significant rises in those on concomitant stable protease inhibitor or non protease drugs (Figure 8B). There is no correlation between a change in the $CD4^+$ T cells count and the dosage of hCG (r=.101, p=.339, N=92) (data not shown).

Among the 26 patients analyzable for weight gain (patients who started hCG preparation therapy coincident with

or shortly after starting other anti-viral therapy were excluded), 14 gained weight, 3 experienced weight loss, and 9 remained stable. There was no correlation between weight change and dosage of hCG (data not shown). There was however a pattern observed in some patients where an initial weight gain was followed by a return to baseline levels while others experienced sustained weight gain over several months.

hCG therapy was well tolerated clinically by patients and there was no evidence for an adverse effect of hCG on viral load or $CD4^+$ T cell level. In two cases with advanced HIV disease hCG was discontinued because of coincident cholestasis probably due to other medications in one case and opportunistic infections in the other.

7.2. EFFECTS OF β-hCG PEPTIDES ON KAPOSI SARCOMA CELLS

Neoplastic Kaposi's Sarcoma tumor cells with a characteristic chromosomal abnormality have been reported (Delli-Bovi et al., 1986, *Cancer Res.* 46:6333-6338; Siegal, et al., 1990, *Cancer* 65:492-498; Popescu et al., 1995, *JNCI* 86:450-454) and provide a model system for studying the *in vitro* effects of hCG on KS cells. In our prior studies employing immune deficient mice injected with KS tumor cells, some commercial preparations of native hCG killed KS tumor cells *in vivo* apparently by inducing apoptosis and inhibiting angiogenesis. *In vitro* tumor cell colonies were also suppressed in clonogenic assays by the hCG preparations (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Nakamura et al., 1988, *Science* 242:426-430; Ensoli et al., 1989, *Science* 243:223-226; Salahuddin et al., 1988, *Science* 242:430-433; Masood, et al., 1984, *AIDS Res. Hum. Retroviruses* 10:969-976). In the current study, experiments were performed to investigate whether certain β-hCG peptides had the same anti-KS effect as native hCG both *in vitro* in clonogenic assays on cultured KS Y-1 cells and *in vivo* in KS tumors induced in nude mice by injection of cultured Kaposi's Sarcoma cells.

Briefly, the KS Y-1 cells were obtained from mononuclear cells isolated from pleural effusion of an AIDS patient with KS in the lungs. After the depletion of T lymphocytes, monocytes/macrophages and fibroblasts using monoclonal antibodies against CD2, CD3, CD4, CD8, CD10 and CD14 membrane antigens and baby rabbit complement, the cells were cultured in the absence of exogenous growth factors to select for transformed cells. Immunological characterization of the KS Y-1 cells showed that CD34, CD31 and endoglin were expressed. Clonogenic assays were performed by seeding the KS Y-1 or KS-SLK cells in methylcellulose (0.8%, v/v), incubating the cells for 10 days in the presence or absence of the hCG, β-hCG or β-hCG peptide preparations and then counting the number of well-formed colonies of triplicate wells formed after seeding with 5 X 10^4 cells.

As shown in Figure 2A and Table 2, the peptides used in the assay at a concentration of (50 nmoles/ml) with the strongest anti-viral effects (peptides of amino acids 45-57 (SEQ ID NO:6), cyclic 44-57, with cysteine substituted at position 44 (SEQ ID NO:26), 109-119 (SEQ ID NO:7), 109-145 (SEQ ID NO:25), and 47-57 linked at the C-terminus by a peptide bond to the N-terminus of 108-119 (SEQ ID NO:32), and 45-57 linked at the C-terminus by a peptide bond to the N-terminus of 109-119 (SEQ ID NO:30)) also had the strongest anti-tumor effects (i.e., anti-KS effect) on the two KS neoplastic cell lines. It is notable that the highly purified hCG heterodimer (CR127 2 use data concentrations of nmoles/ml) was inactive, as in the *in vitro* HIV assay. There was no anti-KS effect with the highly purified α- and β-chains and the α-hCG peptides, and other β-hCG peptides showed little or no inhibition in clonogenic assays (Table 2). Again, the "Scrambled Satellin A1" peptide (SEQ ID NO:36) exhibited activity while the others scrambled peptides did not.

The effects of the peptides on KS tumor cells were also evaluated *in vivo* in the KS mouse model. To induce KS tumors in the mice, 1 x 10^6/ml KS Y-1 cells in 50 μl PBS or saline

were injected subcutaneously into immunodeficient mice (beige-XID-BNX mice). After one week, tumors ranged in size from 2 mm x 3 mm to 3 mm x 5 mm. Methods for detection of apoptosis (from tissue biopsies) were used, as described in Lunardi-Iskandar et al.(1995, *Nature* 375:64-68). Briefly, the samples were stained *in situ* for the presence of cells with DNA fragmentation. Tissue slides from formalin-fixed tumors were treated with terminal deoxynucleotide transferase for extension of DNA ends (hydroxyl 3') and incorporation of digoxigenin-11-dUTP according to the manufacturer's instructions (Oncor, Gaithersburg, MD). Anti-digoxigenin antibody conjugated with the enzyme peroxidase allowed detection of apoptotic cells, which stain brown, whereas viable cells stain blue.

Shown in Figures 2B-E are representative examples of the effects of hCG and the β-hCG peptides on KS Y-1 tumors in mice. One week after injection with the tumor cells, the mice were treated with crude hCG (APL™, Wyeth Ayerst) or with β-chain peptides 45-57 (SEQ ID NO:6) and cyclic 44-57[Cys44] (SEQ ID NO:26). Figures 2B-E show hematoxylin and eosin staining of thin tissue sections of KS Y-1 induced tumors. Compared to the frequent mitotic activity in the controls (Figure 2B), there is evidence of extensive cell death in the tumors of the animals treated with the β-hCG peptides which are comparable to the findings in animals treated with active hCG preparations (Figures 2C-E). Table 2 presents data showing that the β-hCG peptides with an amino acid sequence of 47-57 linked at the C-terminus by a peptide bond to the N-terminus of 108-119 (47-57::108-119; SEQ ID NO:32) and 45-57 linked at the C-terminus by a peptide bond to the N-terminus of 109-119 (45-57::109-119; SEQ ID NO:30) also had significant anti-KS activity. Additionally, β-hCG peptides of amino acid numbers 109-119, 109-145, 47-55 and 48-56 (SEQ ID NOS:7, 25, 20 and 35, respectively) exhibited some anti-KS activity. Other α-hCG and β-hCG peptides showed no activity (Table 2).

As noted above, some AIDS-KS patients treated by intralesional or systemic injection of some preparations of hCG experience regression of tumor lesions of the skin as well as visceral KS (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269). Patients receiving these preparations showed macroscopic regression and flattening of KS lesions. In situ immunostaining specific for apoptosis detection in tumor biopsies showed evidence of apoptosis and/or, histologically, complete absence of the KS tumor after 2-3 weeks of hCG therapy as shown in Figures 2F, G and H, similar to that seen in the experimental mouse model with the active β-hCG peptides. In control KS tumors treated with diluent only or untreated KS tumor tissues (not shown), there was little evidence of cell death (Figure 2F).

Table 2

ACTIVITIES OF hCG AND hCG SUBUNIT PREPARATIONS AND hCG PEPTIDES.

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
		Inhibition			Enhancement
hCG preparations					
APL™	+++	+++	+++	+++	+++
PREGNYL™	++	++	++	++	++
ORGANON	-	ND	-	-	ND
PROFASI™	+	ND	+	+	+
GOLDLINE	+	ND	-	-	-
STERIS™	++	ND	++	ND	++
SHEIN	+	ND	-	-	-
SIGMA	+++	+++	+++	+++	+++
SIGMA[2]	-	ND	-	-	-
CR127	-	ND	-	ND	-
CR1XY17V	-	ND	-	ND	-
CR1XY17B	-	ND	-	-	-
rhCG	-	-	-	-	-
hCG subunits					
α Chain					
αhCG	-	-	-	-	-

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
	Inhibition				Enhancement
rαhCG	-	-	-	-	-
αfp1769A	-	ND	-	ND	-
β Chain					
rβhCG	-	-	-	-	-
βhCG	++	++	++	++	++
Synthetic peptides β-chain hCG					
1. 109-119	+	ND	+	+	+
2. 109-145	+	ND	+	+	+
3. 45-57	++	++	++	++	++
4. Circ 44-57	+++	+++	+++	+++	+++
5. 47-57::108-119	++	++	++	+++	ND
6. 45-57::109-119	++	++	++	++	++
7. 45-57+109-119	++	ND	++	ND	++
8. 41-54	-	-	-	-	-
9. 38-57	-	ND	-	-	-
10. Scrambled 45-57::109-119	-	-	-	-	-
11. Scrambled 45-57	++	ND	++	ND	ND

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
		Inhibition			Enhancement
12. Scrambled circ. 44-57	-	ND	-	ND	-
13. 6-16	-	-	-	-	-
14. 1-20	-	ND	-	ND	ND
15. 20-47	—	ND	—	ND	—
16. 31-50	-	ND	-	ND	-
17. 46-65	+	ND	+	ND	ND
18. 91-112	ND	ND	-	ND	-
19. 93-100	-	ND	-	ND	ND
20. 110-145	ND	ND	-	ND	-
21. 74-95	-	ND	-	ND	-
22. 7-40	+	ND	+	ND	-
23. 57-93	-	ND	-	ND	-
24. 34-39	-	ND	-	ND	ND
25. 123-145	-	ND	-	ND	ND
26. 134-144	-	ND	-	ND	-
27. 100-110	-	ND	-	ND	ND
28. 113-132	ND	ND	-	ND	-
29. 128-145	-	ND	-	ND	-V

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
	Inhibition				Enhancement
30. 37-55	+	+	+	+	+
31. 51-59	-	ND	-	ND	-
32. 48-56	+	+	+	+	+
33. Trimers	-	ND	-	ND	-
Synthetic peptides α-chain hCG					
34. 88-92	-	ND	-	ND	ND
35. 1-15	—	ND	-	-	-
36. 16-30	-	ND	-	ND	-
37. 26-45	-	ND	-	ND	-
38. 41-61	-	ND	-	ND	ND
39. 57-76	-	ND	-	ND	ND
40. 72-92	-	ND	-	ND	-
41. 1-95	-	-	-	-	-

In Table 2, "-" represents less than 10% effect; "+" represents greater than 15% effect; "++" represents greater than 40% effect; "+++" represents greater than 70% effect; and "ND" represents no data available. The "HIV: in vitro" column reports results from assays of the inhibition of HIV-1 replication in vitro (HIV-1 strains and HIV-1 primary isolates). The "HIV transgenic mice" column reports data from the inhibition of HIV RNA and protein expression in HIV-1 transgenic mice. Columns labeled "KS:in vitro" and "KS:in vivo" report on the inhibition of Kaposi's Sarcoma cell growth in vitro in cultured cells and of Kaposi's Sarcoma induced in mice, respectively, as described above. Column 5 provides data on the relative increase of hematopoietic colony cell number in vitro clonogenic assays. The commercial hCG preparations tested were APL™ (Wyeth Ayerst), PREGNYL™ (Organon), ORGANON (a highly purified hCG preparation obtained from Organon), PROFASI™ (Serono), Goldline, STERIS™, and Shein, and two preparations from Sigma, Sigma[1] (GHO) and Sigma[2] (C1063). The hCG preparations CR127 and CR1XY17V are highly purified hCG preparations and CR1XY17B is a mixture of highly purified α-hCG and β-hCG, all three preparations were obtained from the National Institute of Child Health and Human Development (NICHD) at the National Institute of Health (NIH) and the rhCG is recombinant hCG expressed in a mouse cell line (Sigma). For the hCG subunits "αhCG" and "βhCG" are purified native subunits (Sigma); "rαhCG" and "rβhCG" all the recombinant subunits expressed in mouse cells (Sigma); and αfp1769A is purified, native α subunit (NICHD, NIH). The peptide "scrambled A1" has the sequence Cys-Val-Ala-Gln-Pro-Gly-Pro-Gln-Val-Leu-Leu-Val-Leu-Cys (SEQ ID NO:36) and "Scrambled A2" has the sequence Cys-Val-Ala-Gln-Gly-Val-Leu-Pro-Ala-Leu-Pro-Gln-Val-Val-Cys (SEQ ID NO:37). "Scrambled A1/B" has the sequence of β-hCG peptides 45-57 (SEQ ID NO:6) and 109-119 (SEQ ID NO:7) which have been scrambled. "Trimers" is a mixture of tripeptides from the β-hCG sequence of amino acids 45-57: Leu-Gln-Gly, Leu-Gln-Pro, Gln-Gly-Val, Gln-Pro-Val, Gln-Val-Leu, Val-Leu-

Pro, Leu-Pro-Ala, Leu-Pro-Pro, Pro-Ala-Leu, Pro-Pro-Leu, Ala-Leu-Pro, Pro-Leu-Pro, Leu-Pro-Gln, Pro-Gln-Val, Gln-Val-Val, and Val-Val-Cys (SEQ ID NOS: 38-53, respectively). Peptides were synthesized by Dr. N. Ambulos (University of Maryland Biomedicine Center), Becham (CA) or Peptide Technologies Corp. (Gaithersburg, MD).

7.3. DISCUSSION

The discovery of an anti-KS effect of the pregnancy hormone, hCG, was observed *in vivo* in pregnant Bg-nude mice who did not develop KS as did their male litter mates inoculated at the same time with the KS Y-1 KS tumor line. This observation led to clinical trials of intralesional therapy for KS which documented responses in 83% of treated lesions at the higher dose schedule (Gill, P.S., et al., 1996, submitted). We show herein that some patients treated intralesionally with hCG for KS were noted to have a reduction in viral load and *in vitro* and *in vivo* animal model data show that some hCG preparations, partially purified β-hCG, and the active β-hCG fragments (β-hCG peptides 45-57 and 109-119) have anti-KS effects.

We found considerable anti-KS activity with the native partially purified whole β-chain, but recombinant β-hCG (purified) had little or no effect. We suspect that the lower molecular weight species may retain the effect and that some purification procedure may not eliminate those species.

The native hCG and native β-chain preparations available for clinical use are not homogenous and may be contaminated with one or more other active molecules. In this respect, it is noteworthy that though the effects of some preparations of hCG described here were obtained with two different commercial sources of hCG (APL and Pregnyl), one was usually more active (APL) at lower concentrations than any other preparation, although it too varied from lot to lot as detected in the immunodeficient mouse KS system (data not shown) despite the fact that we used identical amounts (International Units) as assessed by the manufacturer's

standard bioassays for the conventional use of hCG. The differences in activities of commercial preparations might be explained by variation in the amount of β-hCG fragments. This could be the consequence of different methods of preparation or different sources of human urine. For example, free β-hCG is more abundant in the earliest weeks of pregnancy. Consequently, we initiated studies with a variety of synthetic peptides, and our results show that all the *in vitro* activities of the preparations of native hCG are mimicked by the β-hCG peptides 45-57, and 109-119 but not other β- or α-peptides or scrambled 45-57 peptide. Thus, we suggest that β-hCG contains structural motifs that produce effects which probably work by mechanisms which differ from those currently known for hCG. We suspect that β-hCG fragments have biological functions quite distinct from the conventional effects of the heterodimer. The structural features of hCG (Lapthorn, A.J., et al., 1994, *Nature* 369:455-461) and appearance in very early pregnancy (Fan, C., et al., 1987, *J. Clin. Endo. Metab.* 64:313-318) combined with some of our observed effects of the β-chain peptides on Kaposi's Sarcoma tumors involving induction of apoptosis (Lunardi-Iskandar, Y., et al., 1995, *Nature* 375:64-68), suggest that the structural similarity to some growth factors may be important and might also be relevant to the hematopoietic growth promoting and anti-viral effects observed here. In view of the evidence that the α subunits are needed for binding to the hCG receptor, we are uncertain how the β peptides initiate these effects. Thus, whether the effects we have observed (anti-viral, anti-tumor, anti-wasting and pro-hematopoietic) are mediated by known hCG receptors is unknown. Given that the mechanism of action of these hCG fragments is likely to involve pathways distinctive from normal hCG hormonal pathways, it is proposed that these active peptides represent a new class of active molecules which we named Satellins. The first members of this class are Satellin A for the active moiety from the β-hCG peptide 45-57 and Satellin B for the β-hCG peptide 109-119.

In laboratory tests, KS cells were killed and regression occurred of transplanted KS tumors in mice (Lunardi-Iskandar, Y., et al., 1995, *Nature* 375:64-68). A recent clinical study of escalating dose by intralesional injection of hCG (APL, Wyeth Ayerst) for cutaneous KS skin lesions, demonstrated tumor regression in a dose-dependent manner with 8% responding at the lowest dose (250 IU three times per week) and 83% at the highest intralesional dose (2000 IU three times per week) (Gill, P.S., et al., 1996, submitted). It is also noteworthy that regression of visceral lesions occurred in 2 KS patients with advanced KS (Hermans, P., et al., 1995, *AIDS. Res. Hum. Retroviruses* S:96).

The clinical data reported herein confirms many of the beneficial effects observed in the laboratory preclinical studies. As discussed above, some preparations of hCG induced partial or complete regression of KS lesions in patients treated intralesionally when the hCG (APL, Wyeth Ayerst) was used at dose levels of 250 to 2000 IU three times per week.

The intrinsic variability of native hCG preparations led to the discovery that certain β-hCG peptides (satellins) reproduce the anti viral and anti KS effects *in vitro* as well as the anti-KS effect in mice with transplanted KS tumors.

8. EXAMPLE: FRACTIONATION OF ACTIVE hCG PREPARATIONS AND HUMAN EARLY PREGNANCY URINE

The present inventors have found that certain commercial preparations of hCG, for example, hCG APL™ (Wyeth-Ayerst), had higher anti-HIV, anti-Kaposi's Sarcoma, anti-wasting and pro-hematopoietic activity than other commercial preparations of hCG (see discussion in section 7 *supra*). Further, the inventors have also shown that highly purified preparations of native and recombinant hCG and β-hCG had no activity against HIV infection or replication or against Kaposi's Sarcoma (see results discussion section 7 *supra*). Accordingly, the inventors postulated that there must be an activity in the hCG commercial preparations that is not the

hCG dimer or the β-hCG subunit, responsible for the anti-HIV, anti-Kaposi's Sarcoma, anti-wasting and pro-hematopoietic activities. This section presents results of the fractionation of the APL™ hCG commercial preparation and urine from women in the first trimester of pregnancy ("human early pregnancy urine") which also contains hCG. Particular sizing column chromatography fractions were shown to have activity, thus demonstrating that the active components could be fractionated.

8.1. MATERIALS AND METHODS

Both human early pregnancy urine and the APL™ (Wyeth-Ayerst) hCG commercial preparation were subjected to fractionation. For the human early pregnancy urine, 5 liters of urine were collected from women in the first trimester of pregnancy. Twenty-four hour collections were stored frozen or refrigerated for up to 2 days. Upon delivery of the urine to the laboratory, sodium azide was added at 1 g/liter and the urine frozen until five liters had been collected. At this time, all the urine was thawed overnight, and the pH was adjusted to 7.2-7.4 with NaOH, which causes some precipitation. The precipitate was allowed to sediment for 1 hour at room temperature, most of the supernatant decanted and the remaining supernatant centrifuged to remove any additional precipitate with that supernatant being added to the first supernatant decanted. Next, the urine was concentrated with a Pellicon (Millipore) filtration system using a membrane cassette with a 3,000 MW cut off, which concentrates the urine approximately 60 to 80 fold. Next, the urine was desalted and delipidated by passing 500 ml of the material at a time through a Sephadex G25 column with a volume of 1.7 liters in 0.05 M ammonium bicarbonate (the column was washed between runs with 25% ethanol to remove absorbed lipids and glycoprotein). The material was lyophilized and stored for further fractionation. The urinary material was then reconstituted in 6 ml of 30 mM sodium phosphate buffer, pH 8.3 fractionation.

For the APL™ hCG, the lyophilized hCG preparation from eleven vials (each vial containing 20,000 IU hCG) was resuspended in 6 ml of 30 mM sodium phosphate buffer, pH 8.3 and filtered twice through the 0.45 μm particle filter. For both the lyophilized urine and the hCG APL™, the prepared sample was then loaded onto a pre-packed SUPERDEX™ 200 HiLoad Column (Pharmacia 26 mm^2 x 60 cm) in the 30 mM sodium phosphate buffer, pH 8.3 and then eluted from the column with a solution containing 30 mM sodium phosphate buffer, pH 7.0 and 2 M NaCl. For the first ten minutes, the column flow rate was 1 ml/minute (due to the viscosity of the hCG APL™ material; this flow rate scheme was also used for the urine material); after the first 10 minutes, the flow rate was 2 ml/minute. The column was run on a Hewlett Packard 1050 HPLC equipped with a photodiode array detector. Four ml fractions were collected and frozen until further analysis.

The protein concentration in each fraction was determined by amino acid analysis. A 50 μl aliquot of alternate column fractions was processed for anlysis by hydrolysis in vapors of 6N HCl with 0.1% phenol at 110°C for 24 hours in a Waters Associates Pico-Tag Workstation (Waters, Milford, MA). An internal standard, norleucine, was added to all fraction samples before hydrolysis to correct for any losses during hydrolysis or liquid transfer. The hydrolyzed samples were then analyzed on a Beckman Instruments 6300 amino acid analyzer and the data was collected on the PE Nelson Data System (Perkin-Elmer) and transformed using PE Nelson Turbochrome software.

The column fractions were monitored with immunoassays to heterodimeric hCG as well as to the hCG beta core fragment (O'Connor et al., 1994, *Endocrin. Rev* 15:650-683; Krichevsky et al., 1994, *Endocrinology* 134:1139-145; Krichevsky et al., 1991, *Endocrinology* 128:1255-1264; O'Connor et al., 1988, *Cancer Res.* 48:1361-1366; Krichevsky, 1988, *Endocrinology* 128:584-593). These two assays permit placement of two internal standard sizes for the gel filtration column: 70,000 kD (hCG) and 10,000 kD (hCG beta core fragment which is amino

acids 6-40 of β-hCG linked via a disulfide bond to amino acids 55-92 of β-hCG). External molecular weight standards were also employed to calibrate the column elution positions. In addition, MALDI-TOF mass spectrometry was used to evaluate the ions observed in certain active fractions. Mass spectrometry did indicate that some peptides separated at anomalous positions, showing that they were being carried by other proteins to earlier elution positions in some cases, or interacting with the column matrix and eluting much later than their molecular size would indicate. For example, 3,000 and 6,000 molecular weight materials eluted from the gel filtration column with material of 14,000 molecular weight while 11,000 molecular weight material eluted with material of approximately 1,000-2,000 molecular weight, hCG and hCG-related molecules eluted at their expected positions.

The fractions were then tested for anti-HIV and anti-KS activities in vitro. To assay for inhibition of HIV-1 replication in vitro, the HIV-1 IIID viral strain (at 10^3 $TCID_{50}$/ml) was used to infect PBMCs and PM-1 cells (derived from the HUT-78 T-cell lymphoma cell line) (10^6 cells/ml) for a 2 hour incubation after which the virus infected cells were washed with 10 ml phosphate buffered saline (PBS) three times to eliminate extracellular virus. The cells were incubated for three days in 100 IU/ml of the hCG APL™ or β-hCG C-Sigma preparations; 50-100 μl per ml of the hCG APL™ or early pregnancy urine fractions; 50 μg/ml β-core protein or α-hCG preparation; 200 IU/ml of the highly purified CR127 hCG preparation; or 100 μl/ml of the circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26). Cultures were then assayed for p24 antigen. The inhibition of HIV production by the active preparations was not due to cell toxicity since, at the concentrations used, there was little or no effect on ^{3}HTdR incorporation, or cell viability as assessed by cell counts and 3-[4,5 dimethylthiazol-2-y], 2,5 diphenyltetrazolium, and bromide thiazoylblue (MTT) assay.

To assay for activity against Kaposi's sarcoma cell growth in vitro, the clonogenic assay described in section 7.2 *supra* was used with the KS Y-1 and KS-SLK cultured Kaposi's Sarcoma cell lines. The cells were incubated in 200 IU/ml of commercial hCG preparations; 50 μl/ml of certain fractions from the hCG preparation of early pregnancy urine fractionation; or 100 μg/ml β- and α-hCG chains, β-hCG core protein, β-hCG peptides or LH (leuteinizing hormone).

Certain fractions were tested for activity in reducing Kaposi's Sarcoma lesions in the Kaposi's Sarcoma mouse model as described in section 7.2 *supra*. In this assay, starting one week after injection of the KS Y-1 cells to induce Kaposi's sarcoma formation, the mice were injected subcutaneously with 100 IU hCG APL™; 200 IU highly purified hCG preparation CR127; 100 μg α-hCG, β-hCG, recombinant β-hCG, LH (luteinizing hormone), or β-core protein; 200 μl of fractions of commercial hCG preparation or early pregnancy urine; or 200 μg cyclized β-hCG peptide of amino acids 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) per day for one week. After one week of the week of treatment with the hCG fractions, the KS lesions were examined for cell apoptosis and regression as described section 7.7 *supra*.

The unfractionated APL™ hCG preparation, PREGNYL™ (Organon) hCG preparation, purified β-core and phenol were also tested in certain assays. Phenol, which is an additive in the hCG APL™ preparation, was tested to control for any effect on cell growth or viral inhibition.

8.2. RESULTS

Fractionation of both the APL™ hCG preparation and the human early pregnancy urine resulted in a significant protein peak at approximately 158 kD with diminishing, but still detectable, protein in the rest of the fractions, even those containing small molecular weight material (Figures 5A and D). Fractions containing the hCG dimer (77 kD) and the β-hCG core (10 kD) were identified by immunoprecipitation using antibodies that specifically recognize these particular

species, as described in the materials and methods section 8.1. The elution profile of the commercial hCG material was also plotted in comparison to the elution of standard molecular weight markers (Figures 9A and B). Additionally, Fractions 61, 63, 64, 65 and 67 from the fractionation of the commercial hCG material was analyzed by MALDI-TOF mass spectrometry (Figures 10A-E, respectively).

8.2.1. EFFECT OF FRACTIONS OF COMMERCIAL hCG PREPARATIONS AND EARLY PREGNANCY URINE ON HIV-1 REPLICATION IN VITRO

The fractions of both the APL™ hCG preparation and the human early pregnancy urine were assayed for inhibition of HIV-1 IIID replication in PBMCs and PM-1 cells as described above. Many of the APL™ hCG preparation fractions exhibited significant inhibition of HIV-1 IIID replication (Figure 5C). In particular, fractions containing material of approximately 70 kD to approximately 2-3 kD exhibited HIV-1 inhibitory activity. The fractions effecting the highest percent inhibition of HIV-1 replication were fractions 62, 63, 65, and 73, with the three main peaks of activity eluting with apparent molecular weights of approximately 40 kD, approximately 15 kD, and approximately 2-3 kD, as determined by comparison with the elution of albumin (158 kD), hCG (77 kD), β-hCG (44 kD) and β-core protein (10 kD).

The fractions of human early pregnancy urine were also assayed for ability to inhibit HIV-1 IIID replication in the PBMCs and the PM-1 cells. Again, several fractions had at least some HIV-1 replication-inhibitory activity. Fractions 64 and 67 caused more than twice the inhibition of HIV-1 IIID replication than any of the other fractions (Figure 5F). There were approximately two peaks of activity eluting from the gel filtration column with apparent molecular weights of approximately 15 kD and 3 kD, as determined by comparison with the elution of albumin (158 kD), hCG (77 D), β-hCG (44 kD), and β-core protein (10 kD) identified by immunoassay.

Additionally, phenol had no effect on HIV-1 replication, demonstrating that the anti-HIV activity of the APL™ hCG is not due to the presence of phenol in the APL™ hCG preparation, and purified β-hCG core protein (the peptide of amino acids to 6-40 of β-hCG linked via a disulfide bond to the peptide of amino acids 55-92 of β-hCG as depicted in Figure 3 (SEQ ID NO:2)) was also found not to inhibit HIV-1 replication (data not shown).

8.2.2. EFFECT OF FRACTIONS OF COMMERCIAL hCG AND EARLY PREGNANCY URINE ON KAPOSI'S SARCOMA CELL GROWTH IN VITRO

The fractions of APL™ hCG and human early pregnancy urine were also tested for inhibition of the proliferation of cultured Kaposi's Sarcoma cells. Figure 5B depicts the results of assays of the APL™ hCG fractions for inhibition of KS Y-1 cell growth. There were three major peaks of KS cell growth inhibitory activity which eluted from the gel filtration column with apparent molecular weights of approximately 40 kD, approximately 15 kD, and approximately 2-3 kD, as compared with the elutions of fractions containing hCG dimer (77 kD) and β-core protein (10 kD). A fraction containing material about the same size as the β-hCG core protein exhibited the highest level of inhibition; however, purified β-hCG core was found not to inhibit KS cell growth (data not shown).

Fractions of human early pregnancy urine were also assayed for inhibition of KS Y-1 cell growth. Fractions containing material which eluted from the gel filtration column with apparent molecular weights of approximately 15 kD and approximately 2-3 kD as compared with the elution of fractions containing hCG dimer (77 kD) and the β-hCG core (10 kD) as identified by immunoprecipitation assay were the most effective at inhibiting KS cell growth, with the approximately 15 kD fractions having the highest activity (Figure 5E).

Figure 6 presents additional data on the inhibitory effects of hCG and hCG-related preparations in KS cultured cell clonogenic assays using both the KS Y-1 and KS SLK assays. Fraction 65 (from the peak eluting with an apparent molecular weight of approximately 15 kD) and 76 (from the peak eluting with an apparent molecular weight of approximately 2-3 kD) from the fractionation of both the APL™ hCG preparation (fraction 65 and 76 are represented by bars 12 and 13, respectively,) and the early pregnancy urine (fraction 65 and 76 are represented by bars 10 and 11, respectively) inhibited growth of both cell lines. The fractions containing material eluting with an apparent molecular weight of approximately 2-3 kD (i.e. fraction 76 of both fractionations) inhibited KS cell growth marginally more effectively than the fractions containing material eluting with an apparent molecular weight of approximately 15 kD (i.e. fraction 65 of both fractionations). Although the active fractions elute close to the fractions containing the β-hCG core protein, purified β-hCG core protein (bar 5) exhibited almost no inhibition of KS cell growth.

The results confirm that the APL™ hCG commercial preparation (bar 1) inhibited KS cell growth better than the other commercial hCG preparations (bars 2-4). Additionally, while native β-hCG (bar 6) inhibited KS cell growth moderately well, α-hCG, the highly purified hCG preparation CR 127 and recombinant hCG (Sigma) (bars 7-9, respectively) inhibit the KS cell growth negligibly. The results also confirm that the cyclized β-hCG peptide of amino acids 44-57 (cysteine substituted at position 44; SEQ ID NO: 26) also inhibited KS cell growth.

8.2.3. EFFECT OF COMMERCIAL hCG AND EARLY PREGNANCY URINE FRACTIONS ON KAPOSI'S SARCOMA IN VIVO

Certain fractions of the APL™ hCG and early pregnancy urine were assayed for their ability to elicit apoptosis in Kaposi's Sarcoma lesions induced by injection of KS Y-1 cells in mice (n=3 mice for each treatment). The mice were

administered 100 μl subcutaneously of the particular fraction each day for one week. Table 3 presents data on the size of the Kaposi's Sarcoma lesions and the percentage of apoptotic cells within the lesion after one week of treatment with fractions 60, 64, 64, 74, 82 and 85 of the APL™ hCG fractions and the unfractionated APL™ hCG preparation. The negative control treated with no hCG or fractionated hCG material exhibited little cell apoptosis or Kaposi's Sarcoma lesion regression (Table 3). Treatment with fractions 82 and 85 (containing material with apparent molecular weights smaller than approximately 2-3 kD) of the APL™ hCG material also elicited almost no Kaposi's Sarcoma lesion regression or apoptosis (Table 3). The unfractionated APL™ hCG, as well as fractions 60 and 74 (fractions within the peaks containing material with apparent molecular weight of approximately 15 kD and 2-3 kD, respectively) of the APL™ hCG fractionated material, caused about 50% apoptosis within the lesion and significant lesion regression (Table 3). Moreover, fractions 64 and 65 (within the peak containing material with apparent molecular weight of approximately 15 kD) of the APL™ hCG showed even higher percentage of apoptosis and more significant lesion regression than the unfractionated APL™ (Table 3).

Additionally, Figure 7 presents results on the effects of certain fractions of the APL™ hCG and the early pregnancy urine on KS tumors induced in mice. Those fractions from the anti-HIV and anti-KS (in vitro) peaks containing material having apparent molecular weight of approximately 15 kD (fraction 65 of the early pregnancy urine ("HAF-UF#") and fractions 62 and 65 of the APL™ hCG preparation ("HAF-CF#")) and of approximately 2-3 kD (fraction 76 of the early pregnancy urine and fractions 74 and 76 of the APL™ hCG preparation) diminished KS tumors in mice as well or better than the unfractionated APL™ hCG ("APL"). However, the fractions tested that were outside these peaks of anti-HIV and anti-KS (in vitro) activities, *i.e.*, fraction 35 of the APL™ hCG (having an apparent molecular weight much larger

than the hCG dimer (77 kD)) and fractions 26 and 82 of the early pregnancy urine (having apparent molecular weights much larger than the hCG dimer and smaller than 2-3 kD, respectively) did not cause tumor regression in the mouse model.

Thus, these results correlate with the results from the HIV replication and KS clonogenic assays, that the activity elutes from the gel filtration column in peaks with apparent molecular weights of approximately 15 kD and 2-3 kD (fractions, with an apparent molecular weight of approximately 44 kD were not assayed).

TABLE 3

Fraction	Kaposi's Sarcoma Lesion Size After Treatment (mm x mm)	Percentage Apoptosis Within Lesion
None	14x10,12x17,13x16	3%,2%,5%
APL#60	4x3,3x2,2x2	>50%
APL#64	1x2,1x3,2x3	>60%
APL#65	2x4,2x3,2x1	>60%
APL#74	3x5,2x5,3x4	>50%
APL#82	15x16,13x19,16x14	2%,4%,6%
APL#85	11x24,13x16,10x13	5%,6%,4%
APL prep	2x3,3x3,3x5	>50%

8.3. CONCLUSION

The above-described experiments demonstrate that the factor(s) responsible for the anti-HIV and anti-KS activities can be further isolated from the hCG preparations by gel filtration on a SUPERDEX™ 200 gel filtration column. The factor(s) were fractionated from both the commercial APL™ hCG preparation and urine from women in early pregnancy (first trimester). The fractions of highest anti-HIV and anti-KS activity contained material eluting from the gel filtration column with an apparent molecular weights of approximately 40 kD, 15 kD and 2-3 kD. Although certain active fractions

contained material of approximately the size of the β-hCG core protein (~10 kD), purified β-hCG core protein was found to have neither anti-HIV nor anti-KS activity. The fractions exhibiting anti-HIV and anti-KS activity in vitro also caused regression of KS tumors induced in mice. Furthermore, phenol, an additive in the APL™ hCG preparation, had no anti-HIV activity.

9. EXAMPLE: EFFECTS OF hCG PREPARATIONS, hCG FRACTIONS AND β-hCG PEPTIDES ON PROSTATE, LUNG, BREAST AND KIDNEY CANCER CELLS

The hCG preparations, fractions of the early pregnancy urine and hCG APL™ SUPERDEX™ 200 fractionations (described in Section 8 *infra*), and certain β-hCG peptides were tested on prostate, lung, breast, and kidney cancer cells *in vitro*, and on prostate tumors in nude mice. The preparations, fractions and peptides were assayed in trypan blue dye assays by seeding cells in liquid culture and then testing for the viability of cells after treatment by trypan blue dye exclusion (viable cells do not stain for trypan blue). The preparations, fractions and peptides were also assayed using a clonogenic assay in which cells were seeded in methylcellulose in the presence or absence of test substance and then colonies were counted after a certain period of time. Cells were also examined for apoptosis by confocal microscopy.

9.1. EFFECT OF hCG PREPARATIONS, hCG FRACTIONS AND β-hCG PEPTIDES ON PROSTATE CANCER CELLS

The effects of hCG preparations, hCG fractions and β-hCG peptides were tested in prostate cancer cells both in vitro and in vivo. Cells were incubated in either 10% fetal bovine serum (FBS) or 3% fetal bovine serum plus hCG preparations, hCG fractions and β-hCG peptides. Table 4 presents data on the percentage of cell death as determined by the trypan blue assay.

Table 4

Treatment	10% FBS	3% FBS
PBS	7%	10%
200 IU hCG APL	38%	48%
500 IU hCG APL	44%	68%
Circ. β-hCG 44[Cys]-57 (200 μg/ml)	29%	39%
Circ. β-hCG 44[Cys]-57 (300 μg/ml)	38%	50%
Urine Fraction 60	43%	62%
Urine Fraction 64	30%	58%
Urine Fraction 74	33%	55%
Urine Fraction 23	9%	13%
Urine Fraction 80	8%	15%
APL Fraction 64	26%	42%
APL Fraction 65	27%	43%
APL Fraction 67	23%	39%
APL Fraction 72	22%	32%
APL Fraction 74	35%	52%
APL Fraction 75	28%	40%
PBS	5%	9%

Table 4 shows that the hCG APL™ preparations, fractions 60, 64 and 74 of the human early pregnancy urine SUPERDEX™ 200 fractionation and fractions 64, 65, 67, 72, 74, and 75 of the hCG APL™ fractionation (see Section 8) and the circularized β-hCG peptide 44-57 (with cysteine substituted for position 44; SEQ ID NO:26), all significantly caused cell death of the prostate cancer cells (all hCG fractions were added at a concentration of 200 μl/ml). Note that fractions 26 and 80 of the early pregnancy urine fractionation, which fractions did not have anti-HIV, anti-KS or pro-hematopoietic activity (see Section 8 *supra*), did not increase prostate cancer cell death.

Figures 11A-H show confocal micrographs of prostate cancer cells treated with an hCG or hCG related preparation and then stained with FITC-labelled actin monoclonal antibody

to visualize the cytoskeleton and propidium iodine to visualize the cell nucleus. Condensation of the nucleus and damage to the cytoskeleton are indications of apoptosis. These micrographs demonstrate increased apoptosis in cells treated with hCG APL™ (Figure 11B), the β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) (Figure 11C); and fraction 64 of the human early pregnancy urine SUPERDEX™ 200 fractionation (described *supra* Section 8) (Figure 11D) compared to controls (Figures 11 A and E-H).

Figure 12 presents data on the inhibition of colony formation in the clonogenic assay. All of the hCG APL™ fractions, as well as 200 IU and 500 IU of the hCG APL™ preparation inhibited prostate cancer cell colony formation as compared to PBS alone.

The circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) (200 μg per day), and the hCG APL™ preparation (100 IU per day) were administered systemically to nude mice in which prostate cancers were induced and also caused apoptosis of the prostate cancer cells *in vivo* (Figures 13A-C).

9.2. EFFECT OF hCG PREPARATIONS, hCG FRACTIONS AND β-hCG PEPTIDES ON LUNG CANCER CELLS

The effects of hCG preparations, hCG fractions and β-hCG peptides were tested in lung cancer cells *in vitro*. Lung cancer cells were incubated with the hCG preparations, hCG fractions and β-hCG peptides in both the trypan blue viability assay and the clonogenic assay. Table 5 presents data on the percentage of cell death as determined by the trypan blue assay.

Table 5

Treatment	% dead cells
PBS	11%
200 IU hCG APL	44%
500 IU hCG APL	66%
200 μg/ml β-hCG 44[Cys]-57	42%
300 μg/ml β-hCG 44[Cys]-57	59%
Urine Fraction 60	53%
Urine Fraction 64	59%
Urine Fraction 74	48%
Urine Fraction 23	13%
Urine Fraction 80	16%
APL Fraction 64	39%
APL Fraction 65	36%
APL Fraction 67	29%
APL Fraction 72	28%
APL Fraction 74	40%
APL Fraction 75	38%

Table 5 shows that the hCG APL™ preparations, fractions 60, 64 and 74 of the human early pregnancy urine SUPERDEX™ 200 fractionation and fractions 64, 65, 67, 72, 74, and 75 of the hCG APL™ fractionation (see Section 8) and the circularized β-hCG peptide 44-57 (with cysteine substituted for position 44; SEQ ID NO:26), all significantly caused cell death of the lung cancer cells. All hCG fractions were added at a concentration of 200 μl/ml. Note that fractions 26 and 80 of the early pregnancy urine fractionation, which fractions did not have anti-HIV, anti-KS or pro-hematopoietic activity (as shown in Section 8 *supra*), did not increase lung cancer cell death. These results are also presented as a bar graph in Figure 14.

Figures 16A-F show confocal micrographs of lung cancer cells treated with the hCG or hCG related preparation and then stained with FITC-labelled actin monoclonal antibody to visualize the cytoskeleton and propidium iodine to visualize

the cell nucleus. Condensation of the nucleus and damage to the cytoskeleton are indications of apoptosis. The micrographs show increased apoptosis in cells treated with hCG APL™ (Figure 16B) and the β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) (Figure 16C) compared to controls (Figures 16A and D-E).

Figures 15A-C present data on the inhibition of colony formation in the clonogenic assay. The hCG APL™, fractions 60, 64 and 74 of the human early pregnancy urine SUPERDEX 200 fractionation and fractions 60, 64, and 74 of the hCG APL™ fractionation all significantly inhibited lung cancer cell colony formation as compared to PBS alone controls (Figures 15A and C). Furthermore, the β-hCG peptides circularized β-hCG 44-57 (with cysteine substituted for position 44; SEQ ID NO:26--"SATA2"), β-hCG 45-57 (SEQ ID NO:6--"SATA1"), and fused β-hCG peptides of 45-57::109-119 (SEQ ID NO:30--"SATAB") also inhibited colony formation of the lung cancer cells as compared to controls (Figures 15A-C).

9.3. EFFECT OF hCG PREPARATIONS, hCG FRACTIONS AND β-hCG PEPTIDES ON BREAST CANCER CELLS

The effects of hCG preparations, hCG fractions and β-hCG peptides were also tested in breast cancer cells *in vitro*. Breast cancer cells were incubated with the hCG preparations, hCG fractions and β-hCG peptides in both the trypan blue viability assay and the clonogenic assay. Table 6 presents data on the percentage of cell death as determined by the trypan blue assay.

Table 6

Treatment	% dead cells
PBS	4%
100 IU hCG APL	34%
APL Fraction 26	3%
APL Fraction 55	13.3%
APL Fraction 65	44%

APL Fraction 76	40%
APL Fraction 82	5.4%
Urine Fraction 26	7%
Urine Fraction 55	12%
Urine Fraction 64	25%
Urine Fraction 65	42%
Urine Fraction 76	44%
Urine Fraction 82	7%
100 ug/ml Circ β-hCG 44 [Cys]-57	42%
Scramble 45-57::109-119	8.6%

Table 6 shows that the hCG APL™ preparations, fractions 60, 64 and 76 of the human early pregnancy urine SUPERDEX™ 200 fractionation and fractions 65 and 76 of the hCG APL™ fractionation (see Section 8) and the circularized β-hCG peptide 44-57 (with cysteine substituted for position 44; SEQ ID NO:26), all significantly caused cell death of the breast cancer cells. All hCG fractions were added at a concentration of 200 µl/ml. The scrambled fused β-hCG peptide of amino acids 45-57 and 109-119 did not cause cell death. Note that fractions 26, 55 and 82 of the early pregnancy urine fractionation and fractions 55 and 82 of the hCG APL™ fractionation, which fractions did not have anti-HIV, anti-KS or pro-hematopoietic activity (see Section 8 *supra*), did not increase breast cancer cell death.

The hCG preparation, hCG fractions and β-hCG peptides were also tested for the inhibition of breast cancer cell colony formation in the clonogenic assay. The data is presented in Table 7.

Table 7

Treatment	% inhibition
PBS	98.3%
hCG-APL-100 IU/ml	50%
APL Fraction 26	0%
APL Fraction 55	12.5%

APL Fraction 65	50.4%
APL Fraction 76	56.3%
Urine Fraction 26	0%
Urine Fraction 55	15.2%
Urine Fraction 65	51%
100 ug/ml Circ β-hCG 44[Cys]-57	45%
Scramble 45-57::109-119	0%

The hCG APL™, fraction 65 of the human early pregnancy urine SUPERDEX™ 200 fractionation and fractions 65 and 76 of the hCG APL™ fractionation, and the circularized β-hCG peptide 44-57 (with cysteine substituted for position 44; SEQ ID NO:26) all significantly inhibited colony formation of the breast cancer cells as compared to PBS alone controls. The scrambled fused peptide of amino acids 45-57 (SEQ ID NO:6) and 109-119 (SEQ ID NO:7) did not significantly inhibit of colony formation. Note that fractions 26 and 55 of the early pregnancy urine fractionation and fractions 26 and 55 of the hCG APL™ fractionation, which fractions did not have anti-HIV, anti-KS or pro-hematopoietic activity (see Section 8 *supra*), did not inhibit breast cancer colony formation.

9.4. EFFECT OF hCG PREPARATIONS ON KIDNEY CANCER CELLS

Two different concentrations of the hCG APL™ preparation were tested for the ability to induce apoptosis in kidney cancer cells. Figures 17A-I show confocal micrographs of kidney cancer cells treated with the hCG APL™ preparation and then stained with FITC-labelled actin monoclonal antibody to visualize the cytoskeleton and propidium iodine to visualize the cell nucleus. Condensation of the nucleus and damage to the cytoskeleton are indications of apoptosis. The micrographs show increased apoptosis in cells treated with 100 IU hCG APL™ (Figures 17D-F) and even higher levels of apoptosis in cultured kidney cancer cells treated with 300 IU hCG APL™ (Figures 17G-I) compared to controls treated only with PBS (Figures 17A-C).

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

SEQUENCE LISTING

(1) GENERAL INFORMATION

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(ii) TITLE OF THE INVENTION: TREATMENT AND PREVENTION OF CANCER BY ADMINISTRATION OF HUMAN CHORIONIC GONADOTROPIN

(iii) NUMBER OF SEQUENCES: 37

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 24-JUN-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: USSN 08/709,948
(B) FILING DATE: 09-SEP-1996

(A) APPLICATION NUMBER: USSN 08/669,681
(B) FILING DATE: 24-JUN-1996

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 539 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 26..520

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AGACAAGGCA GGGGACGCAC CAAGG ATG GAG ATG TTC CAG GGG CTG CTG CTG        52
                            Met Glu Met Phe Gln Gly Leu Leu Leu
                            -20                 -15

TTG CTG CTG CTG AGC ATG GGC GGG ACA TGG GCA TCC AAG GAG CCG CTT       100
Leu Leu Leu Leu Ser Met Gly Gly Thr Trp Ala Ser Lys Glu Pro Leu
    -10                 -5                  1

CGG CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG       148
Arg Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu
                10                  15                  20

GGC TGC CCC GTG TGC ATC ACC GTC AAC ACC ACC ATC TGT GCC GGC TAC       196
Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr
            25                  30                  35

TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT       244
Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
        40                  45                  50

CAG GTG GTG TGC AAC TAC CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC       292
Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu
    55                  60                  65

CCT GGC TGC CCG CGC GGC CTG AAC CCC GTG GTC TCC TAC GCC GTG GCT       340
Pro Gly Cys Pro Arg Gly Leu Asn Pro Val Val Ser Tyr Ala Val Ala
70                  75                  80                  85

CTC AGC TGT CAA TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG       388
Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly
                90                  95                  100

GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC       436
Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp
            105                 110                 115

TCC TCT TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA       484
Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg
        120                 125                 130

CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAAAGGCTTC            530
Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
    135                 140                 145

TCAATCCGC                                                             539
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Glu Met Phe Gln Gly Leu Leu Leu Leu Leu Leu Ser Met Gly
-20                 -15                 -10                 -5

Gly Thr Trp Ala Ser Lys Glu Pro Leu Arg Pro Arg Cys Arg Pro Ile
                  1                   5                  10

Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr
          15                  20                  25

Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val
      30                  35                  40

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg
 45                  50                  55                  60

Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Leu
                 65                  70                  75

Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu
             80                  85                  90

Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu
          95                 100                 105

Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro
     110                 115                 120

Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr
125                 130                 135                 140

Pro Ile Leu Pro Gln
                145
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Val Leu Pro Ala Leu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular, linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Gly Val Leu Pro Ala Leu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gly Val Leu Pro Ala Leu Pro Gln
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Val Leu Pro Ala Leu Pro Gln Val
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Val Leu Pro Ala Leu Pro Gln Val Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg
1 5 10 15

Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Leu Asn Pro Val
20 25 30

Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg
35 40 45

Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp
50 55 60

Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser
65 70 75 80

Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu
85 90 95

Pro Gln

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro
1 5 10 15

Arg Gly Leu Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln
20 25 30

Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp
35 40 45

His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser
50 55 60

Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro
65 70 75 80

Ser Asp Thr Pro Ile Leu Pro Gln
85

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro
1 5 10 15

Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr
20 25 30

Pro Ile Leu Pro Gln
35

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Cys Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
 1               5                  10

          (2) INFORMATION FOR SEQ ID NO:30:

        (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 24 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Thr Cys Asp
 1               5                  10                  15
Asp Pro Arg Phe Gln Asp Ser Ser
            20

          (2) INFORMATION FOR SEQ ID NO:31:

        (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 23 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Leu Gln Gly Val Leu Pro
 1               5                  10                  15
Ala Leu Pro Gln Val Val Cys
            20

          (2) INFORMATION FOR SEQ ID NO:32:

        (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 23 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Leu Thr Cys Asp Asp
 1               5                  10                  15
Pro Arg Phe Gln Asp Ser Ser
            20

          (2) INFORMATION FOR SEQ ID NO:33:

        (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 34 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:
```

```
Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly
 1               5                  10                  15
Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys
            20                  25                  30
Pro Thr
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp
 1               5                  10                  15
Val Arg Phe Glu
            20
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Val Leu Pro Ala Leu Pro Gln Val Val
 1               5
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Cys Val Ala Gln Pro Gly Pro Gln Val Leu Leu Val Leu Cys
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Cys Val Ala Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10 15

WHAT IS CLAIMED IS:

1. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising administering to the subject an amount of a purified protein effective to treat or prevent cancer, said protein comprising one or more portions of the amino acid sequence of β-hCG, a peptide having an amino acid sequence consisting of said one or more portion having anti-cancer activity

2. The method of claim 1 in which the amino acid sequence of at least one of said one or more portions is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-6, 8-24, and 33-35, respectively), as depicted in Figure 3 (a portion of SEQ ID NO:2).

3. The method of claim 1 in which the purified protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2), in which said portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

4. The method of claim 3, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29), as depicted in Figure 3 (SEQ ID NO:2).

5. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising administering to the subject an amount of a purified derivative of a protein, said derivative being effective to treat or prevent cancer, said protein comprising one or more portions of the amino acid sequence of β-hCG, a peptide having an amino acid sequence consisting of said one or more portions having anti-cancer activity

6. The method of claim 5 in which the amino acid sequence of at least one of said one or more portions is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25, and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2).

7. The method of claim 5 in which the purified derivative comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2), in which said portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

8. The method of claim 7, in which the amino acid sequence of said derivative is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino

acids 108-119 (SEQ ID NO:29), as depicted in Figure 3 (SEQ ID NO:2).

9. The method of claim 1 or 5 in which the subject is a human.

10. The method of claim 1 or 5 in which the amino acid sequence of the protein consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 3 (a portion of SEQ ID NO:2).

11. The method of claim 1 in which the protein is N-acetylated and has a C-terminal amide.

12. The method of claim 5 in which the derivative contains one or more D-amino acids or one or more non-classical amino acids.

13. The method of claim 1 or 5 in which the cancer is Kaposi's Sarcoma.

14. The method of claim 13 in which the protein is administered by intralesional injection or by intralesional and intramuscular injection.

15. The method of claim 13 which further comprises administering chemotherapy to the subject.

16. The method of claim 1 or 5 in which the cancer is selected from the group consisting of a prostate cancer, a lung cancer, a breast cancer, a kidney cancer, and a pancreatic cancer.

17. The method of claim 16 which further comprises administering chemotherapy to the subject.

18. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising administering to the subject an amount of a circularized protein effective to treat or prevent cancer, the amino acid sequence of which consists of one or more portions of the sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, said circularized protein being active to treat or prevent cancer.

19. The method of claim 18 in which said at least one of said one or more portions has an amino acid sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2).

20. The method of claim 18 in which the circularized protein has an amino acid sequence which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 3 (a portion of SEQ ID NO:2), with cysteine substituted for valine at position 44.

21. The method of claim 18 in which the circularized protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2), in which said portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

22. The method of claim 21, in which the first portion consists of β-hCG amino acid numbers 45-57 (SEQ ID NO:6) and the second portion consists of β-hCG amino acid numbers 110-119 (SEQ ID NO:27) as depicted in Figure 3 (SEQ ID NO:2); and in which a disulfide bond is formed between the cysteine residues at a amino acids 57 and 110 of said portions.

23. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising administering to the subject an amount of a purified protein effective to treat or prevent cancer, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2) in which one or more residues in at least one of said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said protein being active to treat or prevent cancer.

24. The method of claim 23 in which said at least one portion has a sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2).

25. The method of claim 23 in which said at least one portion of said sequence consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 3 (a portion of SEQ ID NO:2), and in which substitutions by said amino acid or amino acid analog occur at residues 47 and 51 of said portion.

26. The method of claim 23 in which one or more residues are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric

acid residue is peptide bonded to a sequence of one or more proline residues.

27. The method of claim 25 in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

28. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising administering to the subject an amount of a circularized protein effective to treat or prevent cancer, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, and also in which one or more residues in at least one of said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said circularized protein being active to treat or prevent cancer.

29. The method of claim 28 in which the circularized protein has an amino acid sequence which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 3 (a portion of SEQ ID NO:2), with cysteine substituted for valine at position 44, and in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue and the side chain amino group of

said diaminobutyric acid residue is peptide bonded to a proline residue.

30. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising administering to the subject an amount of a purified protein effective to treat or prevent cancer, which protein (a) comprises a β-hCG amino acid sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2) and; (b) lacks β-hCG amino acids contiguous to said sequence.

31. The method of claim 30 in which the amino acid sequence consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 3 (a portion of SEQ ID NO:2).

32. The method of claim 30 or 31 in which the subject is a human.

33. The method of claim 30 in which the protein is a fusion protein, said fusion protein comprising the β-hCG amino acid sequence joined via a peptide bond to a protein sequence of a protein different from β-hCG.

34. The method of claim 30 in which the cancer is Kaposi's Sarcoma.

35. The method of claim 34 in which the protein is administered by intralesional injection or intralesional and intramuscular injection.

36. The method of claim 30 in which the cancer is selected from the group consisting of a prostate cancer, a

lung cancer, a breast cancer, a kidney cancer, and a pancreatic cancer.

37. The method of claim 34 or 36 which further comprises administering chemotherapy to the subject.

38. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising administering to the subject an amount of a first composition effective to treat or prevent cancer, said first composition comprising one or more first components of a second composition comprising a sample of native hCG or native β-hCG, said first composition being separated from other components of the hCG or β-hCG sample, said first components having anti-cancer activity, and said second composition having anti-cancer activity, and said native hCG or native β-hCG not being purified to homogeneity in said second composition.

39. The method of claim 38 in which said first components are separated from said other components by sizing column chromatography.

40. The method of claim 39 in which said sizing column chromatography is performed using a SUPERDEX™ 200 column.

41. The method of claim 39 in which said first components have an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

42. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising

administering to the subject an amount of a first composition effective to treat or prevent cancer, said first composition produced by a process comprising the following steps:

(a) subjecting a second composition comprising native hCG or native β-hCG, said second composition having anti-cancer activity, said native hCG or native β-hCG not being purified to homogeneity in said second composition, to a size fractionation procedure; and

(b) recovering fractions having anti-cancer activity.

43. The method of claim 42, in which the recovered fractions contain material having an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

44. A method for screening a preparation comprising a purified protein having a sequence of one or more portions of β-hCG or a purified derivative of said protein or a fraction of a source of native hCG or native β-hCG, for anti-cancer activity comprising assaying said preparation for the ability to inhibit the survival or proliferation of malignant cells.

45. The method of claim 44 in which the preparation is screened by a method comprising measuring the survival or proliferation of malignant cells, which cells have been contacted with the preparation; and comparing the survival or proliferation of the cells contacted with the preparation with the survival or proliferation of cells not so contacted with the preparation, wherein a lower level of survival or proliferation in said contacted cells indicates that the preparation has anti-cancer activity.

46. The method of claim 44 in which the preparation is screened by a method comprising measuring the survival or proliferation of cells from a cell line which is derived from or displays characteristics associated with a malignant disorder, which cells have been contacted with the preparation; and comparing the survival or proliferation in the cells which have been contacted with the preparation with said survival or proliferation in cells not so contacted, wherein a lower level in said contacted cells indicates that the preparation has anti-tumor activity.

47. A method for screening a preparation comprising a purified prctein having a sequence of one or more portions of β-hCG or a purified derivative of said protein or a fraction of a source of native hCG or native β-hCG, for anti-cancer activity comprising assaying said preparation for the ability to convert cells having an abnormal phenotype to a more normal cell phenotype.

48. The method of claim 47 in which the preparation is screened by a method comprising assessing the phenotype of cells suspected of being pre-neoplastic in culture, which cells have been contacted with the preparation; and comparing the phenotype in the cells which have been contacted with the preparation with said phenotype in cells not so contacted, wherein a more normal phenotype in said contacted cells indicates that the preparation has anti-cancer activity.

49. The method of claim 47 in which the preparation is screened by a method comprising assessing the phenotype of cells from a cell line which is derived from or displays characteristics associated with a pre-malignant disorder, which cells have been contacted with the preparation; and comparing the phenotype in the cells which have been contacted with the preparation with said phenotype in cells not so contacted, wherein a more normal phenotype in said

contacted cells indicates that the preparation has anti-cancer activity.

50. A method for screening a preparation comprising a purified protein having a sequence of one or more portions of β-hCG or a purified derivative of said protein or a fraction of a source of native hCG or native β-hCG, for activity in treatment or prevention of Kaposi's Sarcoma comprising assaying said preparation for the ability to inhibit Kaposi's Sarcoma cell proliferation or promote Kaposi's Sarcoma cell apoptosis.

51. The method of claim 50 in which the preparation is screened by a method comprising measuring proliferation or colony formation in cultured KS Y-1 or KS-SLK cells, which cells have been contacted with the preparation; and comparing the measured proliferation or colony formation in the cells which have been contacted with the preparation with said proliferation or colony formation in cells not so contacted with the preparation, wherein a lower level of proliferation or colony formation in said contacted cells indicates that the preparation has anti-Kaposi's Sarcoma activity.

52. The method of claim 50 in which the preparation is screened by a method comprising measuring apoptosis in a Kaposi's Sarcoma tumor in an immunodeficient mouse, which Kaposi's Sarcoma tumors have been induced by injection with KS Y-1 or KS-SLK cells, and which mouse has been exposed to the preparation; and comparing the degree of apoptosis in the tumor of the mouse which has been exposed to the preparation with a tumor in a mouse not so exposed, wherein a higher in level of apoptosis in the tumor of said exposed mouse indicates that the preparation has anti-Kaposi's Sarcoma activity.

53. A pharmaceutical composition comprising a therapeutically effective amount of a purified protein

effective to treat or prevent cancer, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-6, 8-24, and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2); and a pharmaceutically acceptable carrier.

54. The pharmaceutical composition of claim 53 in which the amino acid sequence of the protein consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 3 (a portion of SEQ ID NO:2).

55. A pharmaceutical composition comprising a therapeutically effective amount of a purified protein effective to treat or prevent cancer, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion; and a pharmaceutically acceptable carrier.

56. The pharmaceutical composition of claims 53 or 55 which is formulated as a controlled release formulation.

57. The pharmaceutical composition of claim 55, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus

of β-hCG amino acids 108-119 (SEQ ID NO:29), as depicted in Figure 3 (SEQ ID NO:2).

58. The pharmaceutical composition of claim 55 in which the protein is a fusion protein, said fusion protein comprising at least one of said portions of the β-hCG amino acid sequence joined via a peptide bond to a sequence of a protein different from β-hCG.

59. A pharmaceutical composition comprising a therapeutically effective amount of a purified protein effective to treat or prevent cancer, which protein (a) comprises a β-hCG amino acid sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-6, 8-24, and 33-35, respectively), as depicted in Figure 3 (a portion of SEQ ID NO:2); and (b) a pharmaceutically acceptable carrier.

60. The pharmaceutical composition of claim 59 which is formulated as a controlled release formulation.

61. The method of claim 59 in which the protein is N-acetylated and has a C-terminal amide.

62. A pharmaceutical composition comprising a therapeutically effective amount of a derivative of a protein, said derivative being effective to treat or prevent cancer, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2); and a pharmaceutically acceptable carrier.

63. The method of claim 62 in which the derivative contains one or more D-amino acids or non-classical amino acids.

64. The pharmaceutical composition of claim 62 which is formulated as a controlled release formulation.

65. A pharmaceutical composition comprising a therapeutically effective amount of a derivative of a protein, said derivative being effective to treat or prevent cancer, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, said derivative being active to treat or prevent cancer; and a pharmaceutically acceptable carrier.

66. The pharmaceutical composition of claim 65, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29) as depicted in Figure 3 (SEQ ID NO:2).

67. A pharmaceutical composition comprising a therapeutically effective amount of a circularized protein effective to treat or prevent cancer, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a

different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said said one or more portions of said sequence, said circularized protein being active to treat or prevent cancer; and a pharmaceutically acceptable carrier.

68. The pharmaceutical composition of claim 67 in which at least one of said one or more portions has an amino acid sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2).

69. The pharmaceutical composition of claim 67 in which the circularized protein has an amino acid sequence which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 3 (a portion of SEQ ID NO:2), with cysteine substituted for valine at position 44.

70. The pharmaceutical composition of claim 67, in which the amino acid sequence of said circularized protein consists of two or more at least five amino acid, non-naturally contiguous portions of the β-hCG sequence as depicted in Figure 3 (SEQ ID NO:2) in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

71. The pharmaceutical composition of claim 70, in which the first portion consists of β-hCG amino acid numbers 45-57 (SEQ ID NO:6) and the second portion consists of β-hCG amino acid numbers 110-119 (SEQ ID NO:27) as depicted in Figure 3 (SEQ ID NO:2); and in which a disulfide bond is

formed between the cysteine residue at amino acids 57 and 110 of said portions.

72. A pharmaceutical composition comprising a therapeutically effective amount of a purified protein, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2) in which one or more residues in at least one of said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said protein being active to treat or prevent cancer; and a pharmaceutically acceptable carrier.

73. The pharmaceutical composition of claim 72 in which said at least one portion of said sequence consists of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25, and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2).

74. The pharmaceutical composition of claim 72 in which said portion of said sequence consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 3 (a portion of SEQ ID NO:2), and in which substitutions by said amino acid or amino acid analog occur at residues 47 and 51 of said portion.

75. The pharmaceutical composition of claim 72 in which said one or more residues are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a sequence of one or more proline residues.

76. The pharmaceutical composition of claim 74 in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

77. A pharmaceutical composition comprising an amount effective for treatment of a cancer of a circularized protein, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, and also in which one or more residues in at least one of said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said circularized protein being active to treat or prevent cancer; and a pharmaceutically acceptable carrier.

78. The pharmaceutical composition of claim 77 in which said circularized protein has an amino acid sequence consisting of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 3 (a portion of SEQ ID NO:2), and in which cysteine is substituted for valine at position 44, and in which the residues at positions 47 and 51 of said portion(s) are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

79. A pharmaceutical composition comprising a therapeutically effective amount of a purified nucleic acid encoding a protein effective to treat or prevent cancer, the amino acid sequence of which protein is selected from the group consisting of 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 3 (a portion of SEQ ID NO. 2); and a pharmaceutically acceptable carrier.

80. A pharmaceutical composition comprising a therapeutically effective amount of a purified nucleic acid encoding a protein effective to treat or prevent cancer, the amino acid sequence of which comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion; and a pharmaceutically acceptable carrier.

81. The pharmaceutical composition of claim 80, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29), as depicted in Figure 3 (SEQ ID NO:2).

82. The pharmaceutical composition of claim 79 or 80 in which the nucleic acid is a nucleic acid vector.

83. A pharmaceutical composition comprising a recombinant cell containing a nucleic acid vector encoding a protein effective to treat or prevent cancer, the amino acid sequence of which protein is selected from the group consisting of 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 3 (a portion of SEQ ID NO. 2); and a pharmaceutically acceptable carrier.

84. A pharmaceutical composition comprising a recombinant cell containing a nucleic acid vector encoding a protein comprising two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2), said portions being linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion; and a pharmaceutically acceptable carrier.

85. A pharmaceutical composition comprising (a) a therapeutically effective amount of a purified nucleic acid encoding a protein, which protein (i) comprises a β-hCG amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 3 (a portion of SEQ ID NO:2), and (ii) lacks β-hCG amino acids contiguous to said sequence; and (b) a pharmaceutically acceptable carrier.

86. The pharmaceutical composition of claim 85 in which the nucleic acid is a nucleic acid vector.

87. A pharmaceutical composition comprising (a) a recombinant cell containing a nucleic acid vector encoding a

protein, which protein (i) comprises a β-hCG amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 3 (a portion of SEQ ID NO:2), and (ii) lacks β-hCG amino acids contiguous to said sequence; and (b) a pharmaceutically acceptable carrier.

88. The pharmaceutical composition of claim 87 in which the nucleic acid vector encodes a protein, the amino acid sequence of which protein consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 3 (a portion of SEQ ID NO:2).

89. A pharmaceutical composition comprising a therapeutically effective amount of a purified nucleic acid encoding a derivative of a protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 3 (a portion of SEQ ID NO. 2), said derivative being active to inhibit HIV infection of replication; and a pharmaceutically acceptable carrier.

90. The pharmaceutical composition of claim 89 in which the nucleic acid is a nucleic acid vector.

91. A pharmaceutical composition comprising a recombinant cell containing a nucleic acid vector encoding a derivative of a protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-

58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 3 (a portion of SEQ ID NO. 2), said derivative being active to treat or prevent cancer; and a pharmaceutically acceptable carrier.

92. A pharmaceutical composition comprising a therapeutically effective amount of a purified nucleic acid encoding a derivative of a protein, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 3 ((SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, said derivative being active to treat or prevent cancer; and a pharmaceutically acceptable carrier.

93. The pharmaceutical composition of claim 92 in which the nucleic acid is a nucleic acid vector.

94. A pharmaceutical composition comprising a recombinant cell containing a nucleic acid vector encoding a derivative of a protein, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contigous portions of the amino acid sequence of β-hCG as depicted in Figure 3 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, said derivative being effective to treat or prevent cancer; and a pharmaceutically acceptable carrier.

95. A pharmaceutical composition comprising a therapeutically effective amount of a first composition effective to treat or prevent cancer comprising one or more first components of a second composition comprising a sample of native hCG or native β-hCG, said first components being separated from other components of the hCG or β-hCG sample,

said first components having anti-cancer activity, and said second composition having anti-cancer activity, and said native hCG or native β-hCG not being purified to homogeneity in said second composition; and a pharmaceutically acceptable carrier.

96. The pharmaceutical composition of claim 95 in which said first components are separated from said other components by sizing column chromatography.

97. The pharmaceutical composition of claim 96 in which said sizing column chromatography is performed using a Superdex 200 column.

98. The pharmaceutical composition of claim 96 in which said first components have an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

99. A pharmceutical composition comprising a therapeutically effective amount of a first composition effective to treat or prevent cancer, said first composition produced by a process comprising the following steps:

(a) subjecting a second composition comprising native hCG or native β-hCG, said second composition having anti-cancer activity, said native hCG or native β-hCG not being purified to homogeneity in said second composition, to a size fractionation procedure; and

(b) recovering fractions having anti-cancer activity;

and a pharmaceutically acceptable carrier.

100. The pharmaceutical composition of claim 99, in which the recovered fractions contain material having an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

101. The pharmaceutical composition of claim 99, in which the sample of hCG is early pregnancy urine.

102. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising administering to the subject an amount of a nucleic acid encoding a protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2), active to treat or prevent cancer.

103. A method of treating or preventing cancer in a subject in need of such treatment or prevention comprising administering to the subject an amount of a recombinant cell containing a nucleic acid encoding a protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively) as depicted in Figure 3 (a portion of SEQ ID NO:2), active to treat or prevent cancer.

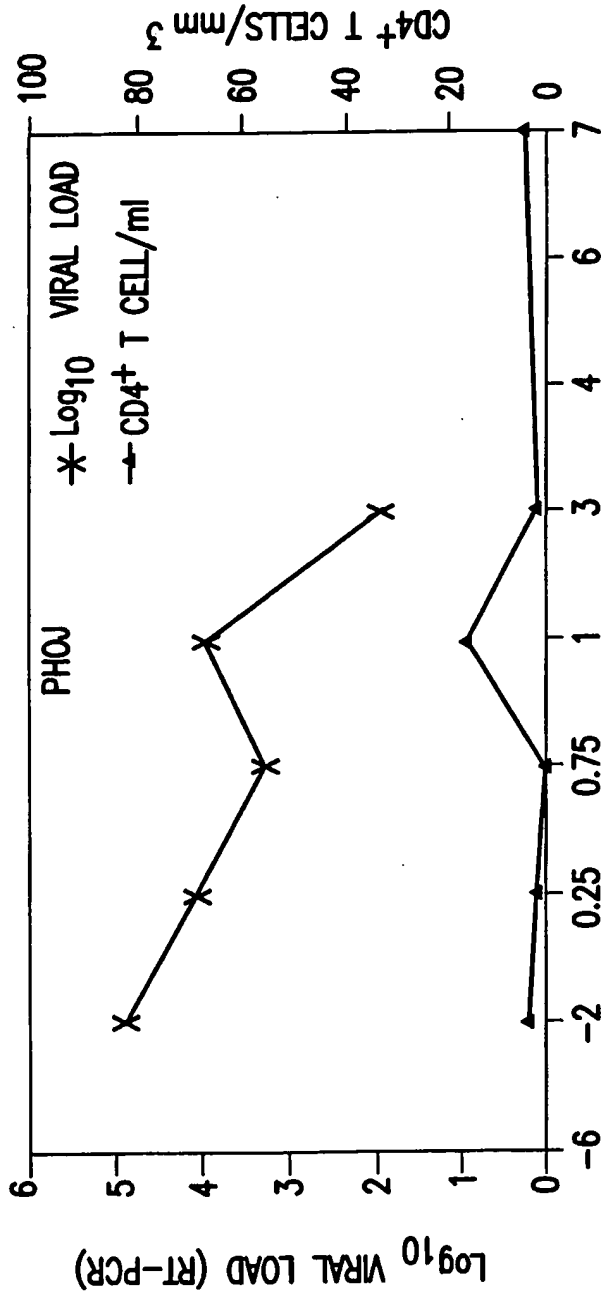

PHOJ
* Log10 VIRAL LOAD
+ CD4+ T CELL/ml
Log10 VIRAL LOAD (RT-PCR)
CD4+ T CELLS/mm 3
0
1
2
3
4
5
6
0
20
40
60
80
100
-6
-2
0.25
0.75
1
3
4
6
7

FIG. 1A

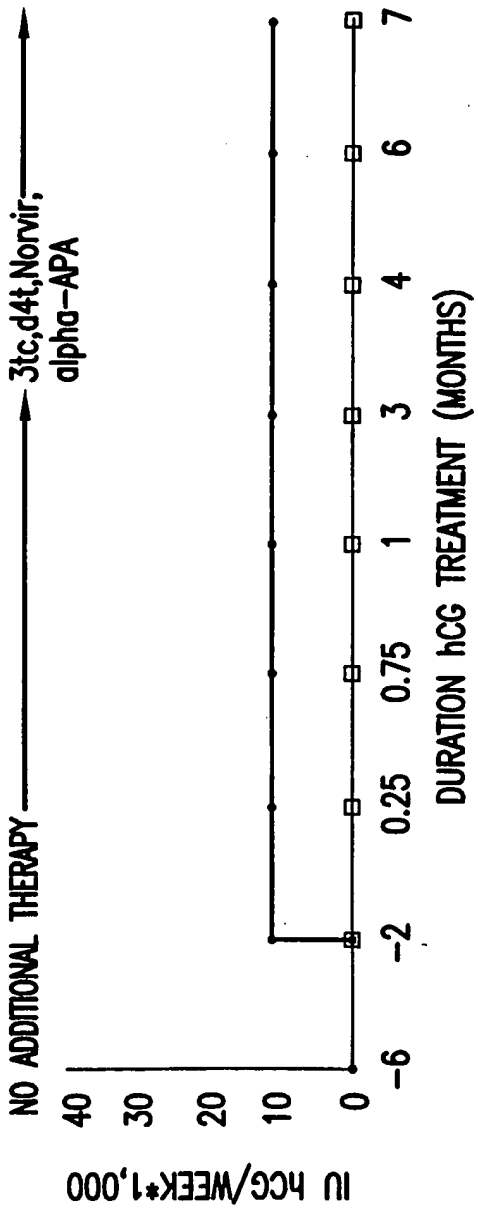

NO ADDITIONAL THERAPY
3tc,d4t,Norvir, alpha-APA
IU hCG/WEEK*1,000
DURATION hCG TREATMENT (MONTHS)
0
10
20
30
40
-6
-2
0.25
0.75
1
3
4
6
7

FIG. 1B

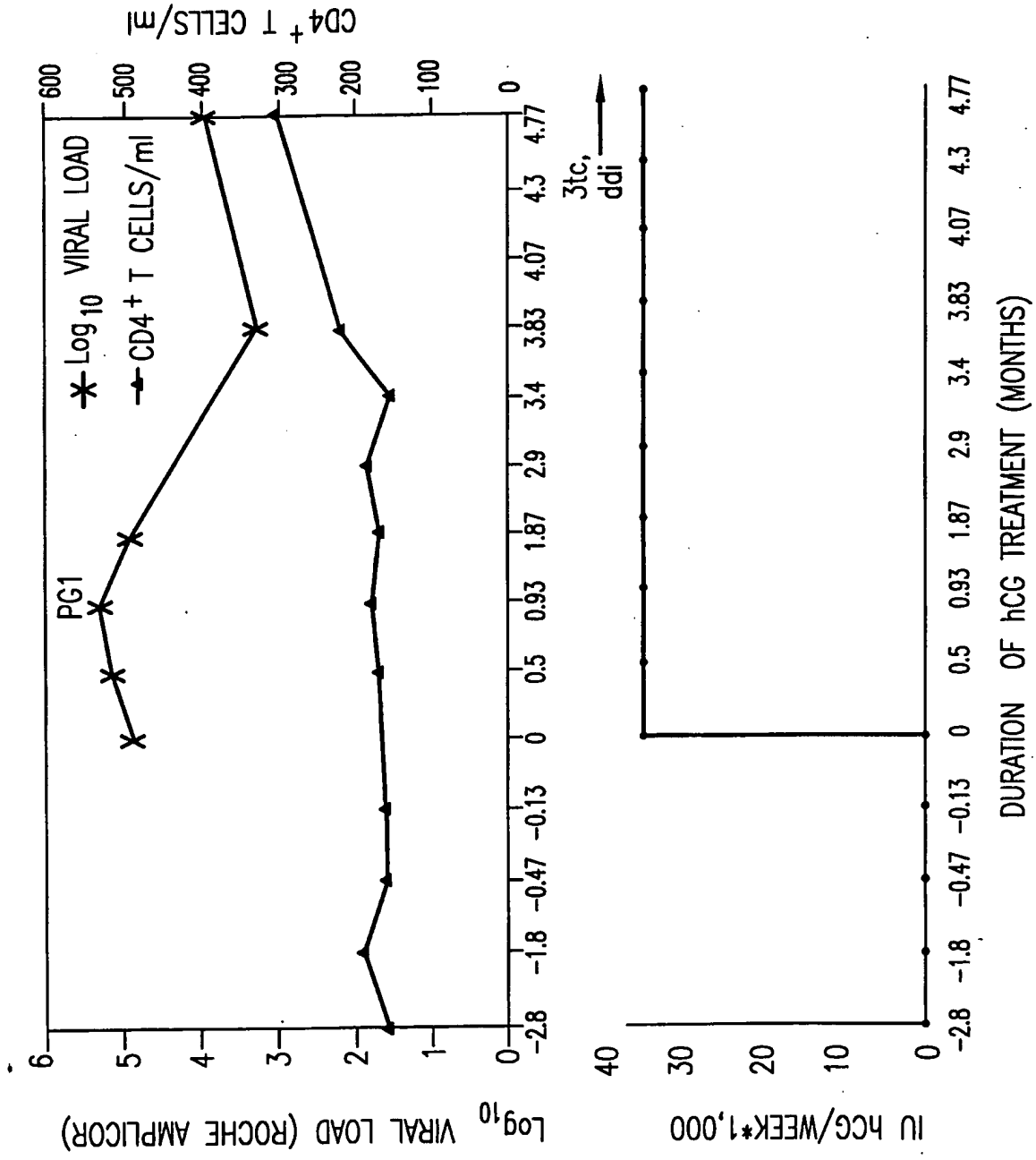

CD4+ T CELLS/ml
600
500
400
300
200
100
0
Log10 VIRAL LOAD
CD4+ T CELLS/ml
PG1
6
5
4
3
2
1
0
-2.8 -1.8 -0.47 -0.13 0 0.5 0.93 1.87 2.9 3.4 3.83 4.07 4.3 4.77
Log10 VIRAL LOAD (ROCHE AMPLICOR)
3tc, ddi
40
30
20
10
0
IU hCG/WEEK*1,000
DURATION OF hCG TREATMENT (MONTHS)

FIG.1C

FIG.1D

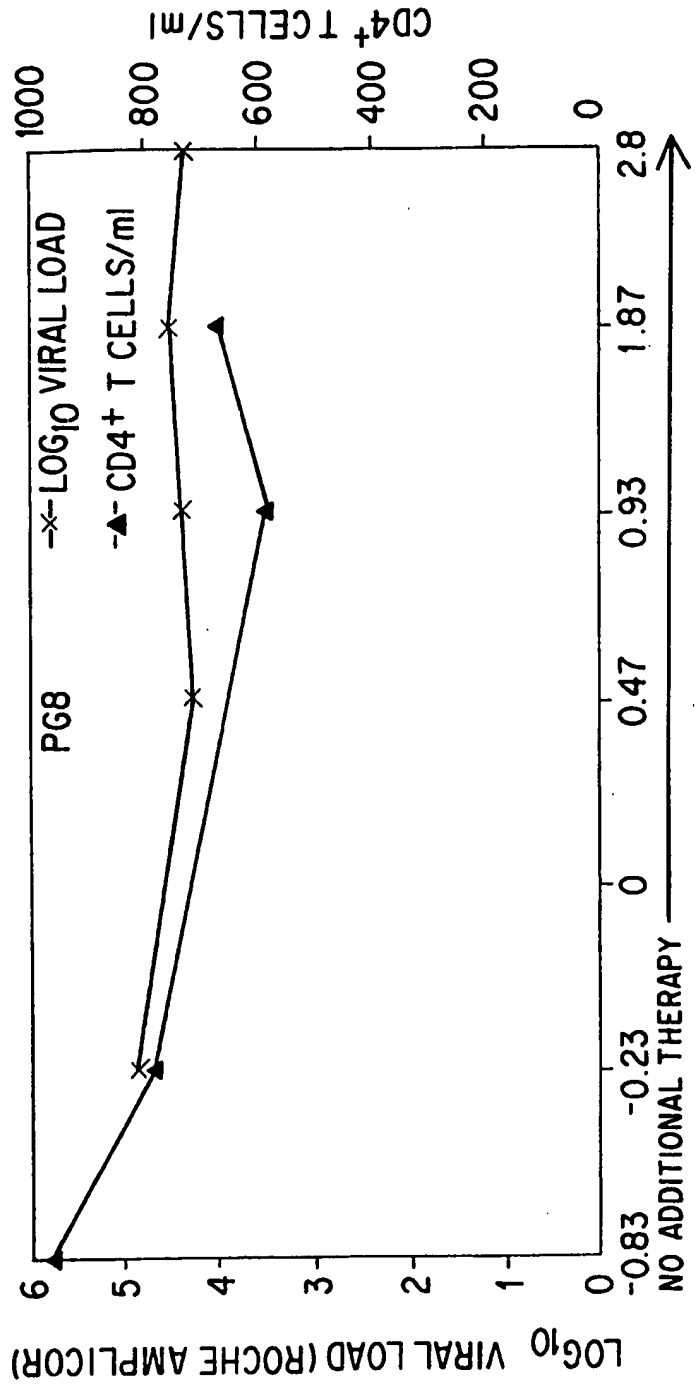

CD4+ T CELLS/ml
1000
800
600
400
200
0
PG8
-×- LOG10 VIRAL LOAD
-▲- CD4+ T CELLS/ml
6
5
4
3
2
1
0
LOG10 VIRAL LOAD (ROCHE AMPLICOR)
-0.83
-0.23
0
0.47
0.93
1.87
2.8
NO ADDITIONAL THERAPY

FIG. 1E

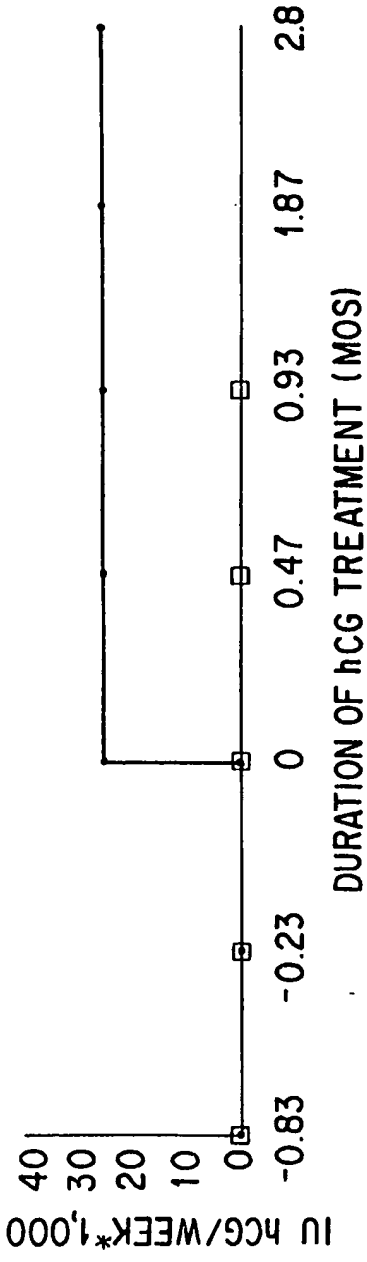

IU hCG/WEEK*1,000
40
30
20
10
0
-0.83
-0.23
0
0.47
0.93
1.87
2.8
DURATION OF hCG TREATMENT (MOS)

FIG. 1F

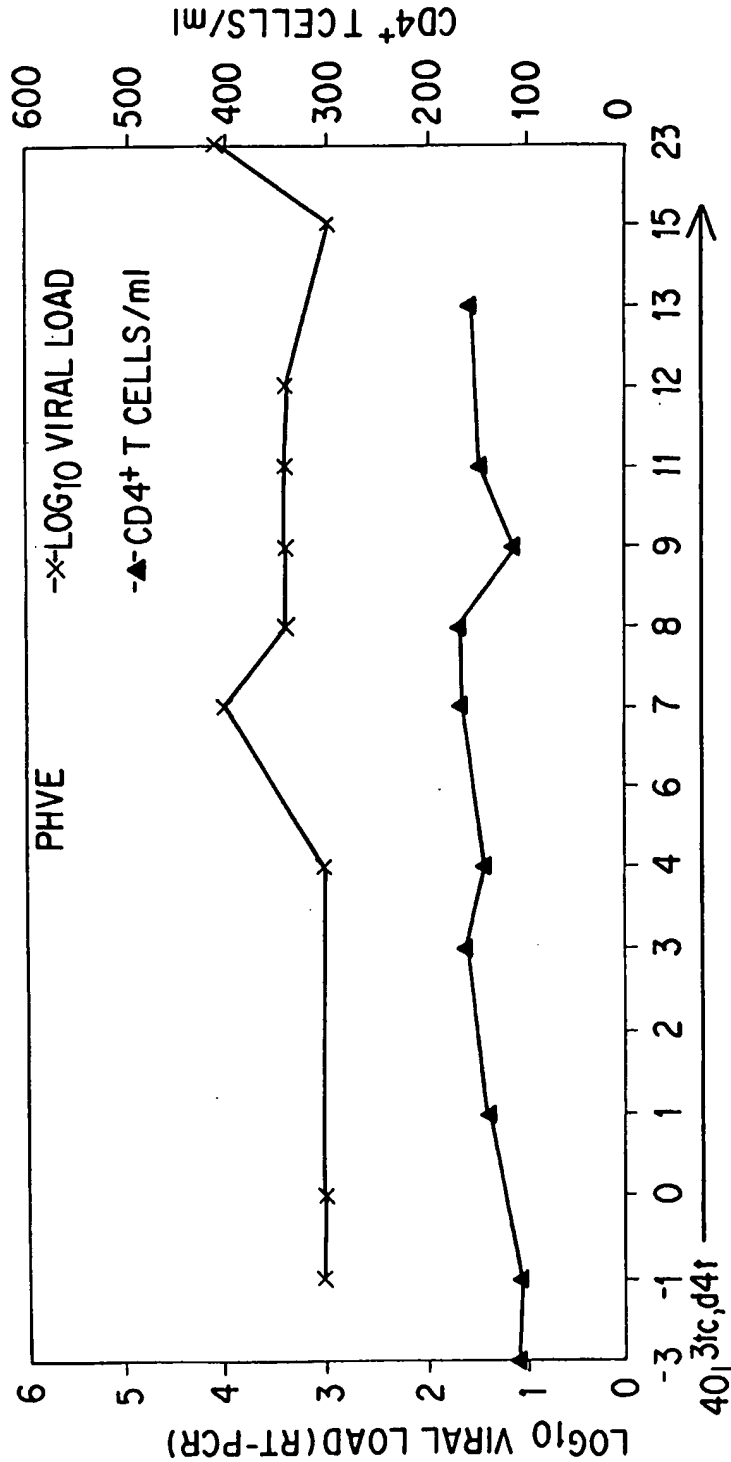

PHVE
-×-LOG10 VIRAL LOAD
-▲-CD4+ T CELLS/ml
LOG10 VIRAL LOAD (RT-PCR)
CD4+ T CELLS/ml
3tc,d4t

FIG.1G

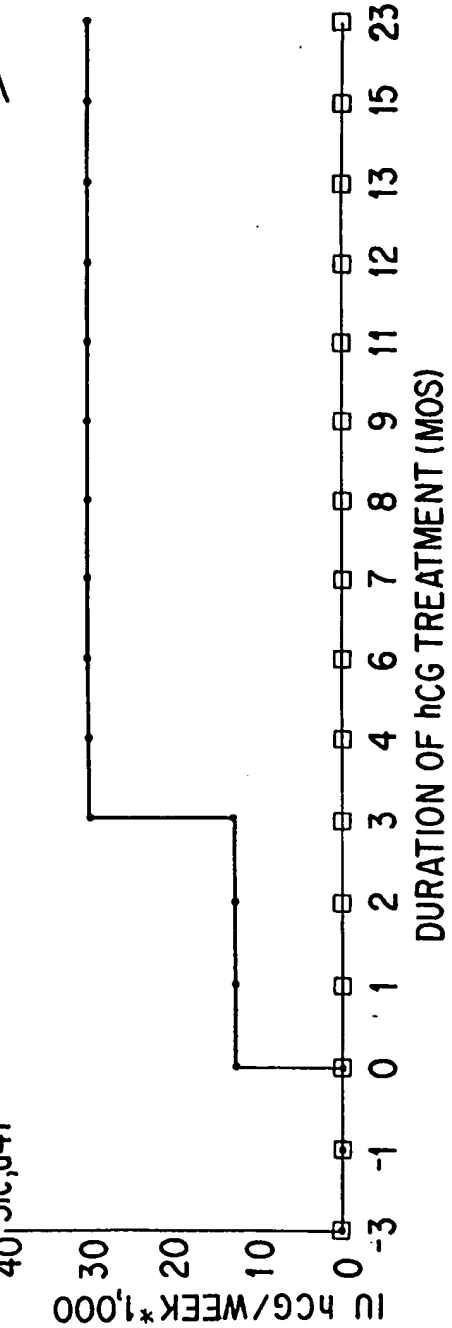

IU hCG/WEEK*1,000
DURATION OF hCG TREATMENT (MOS)

FIG.1H

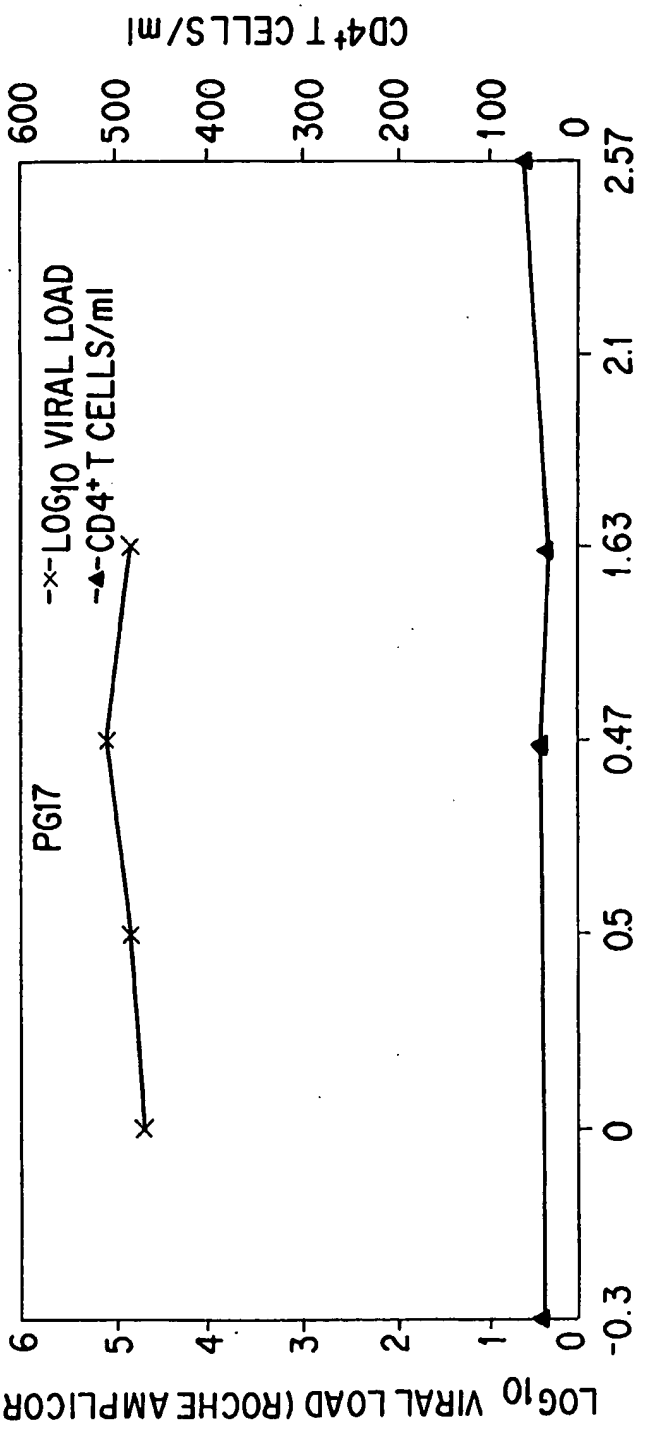

CD4+ T CELLS/ml
600
500
400
300
200
100
0
2.57
2.1
1.63
0.47
0
0.5
-0.3
-x- LOG10 VIRAL LOAD
-▲- CD4+ T CELLS/ml
PG17
NO ADDITIONAL THERAPY
LOG10 VIRAL LOAD (ROCHE AMPLICOR)
6
5
4
3
2
1
0

FIG. 1I

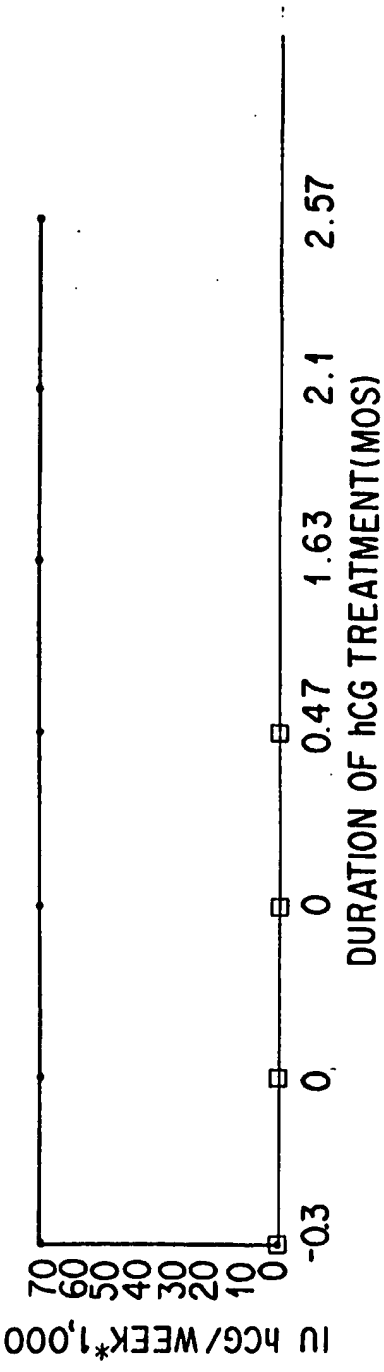

IU hCG/WEEK*1,000
70
60
50
40
30
20
10
0
-0.3
0
0.47
1.63
2.1
2.57
DURATION OF hCG TREATMENT(MOS)

FIG. 1J

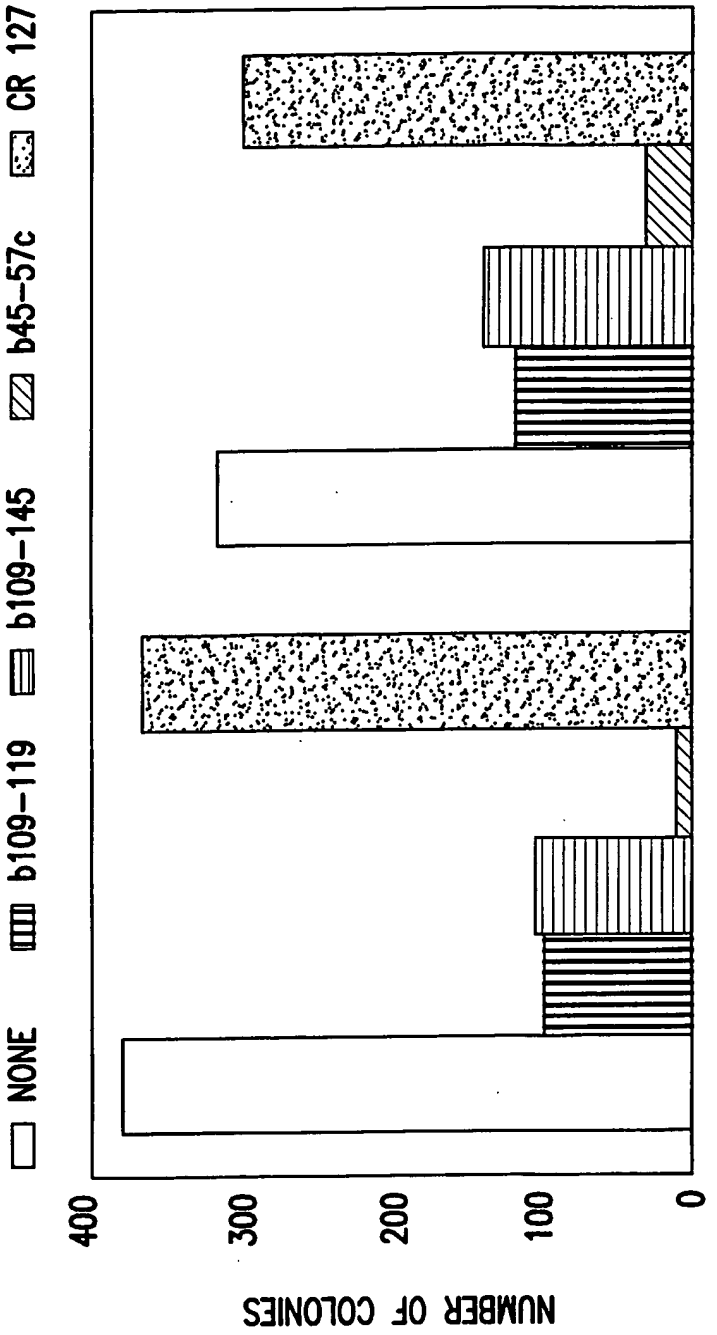

NONE
b109-119
b109-145
b45-57c
CR 127
400
300
200
100
0
NUMBER OF COLONIES
KS Y-1
KS "SLK"
KS CELL LINES

FIG.2A

FIG.2C

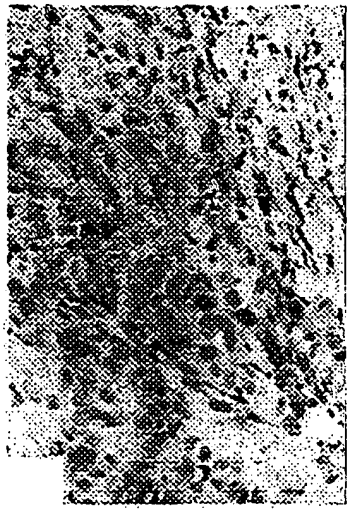


FIG.2E

FIG.2B

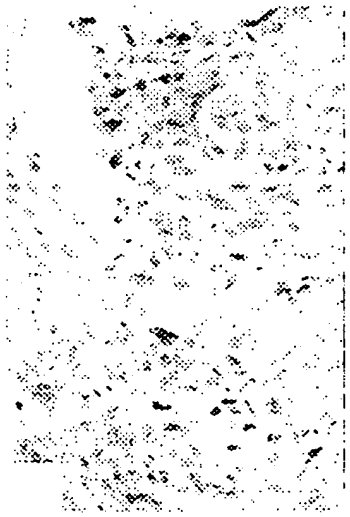


FIG.2D

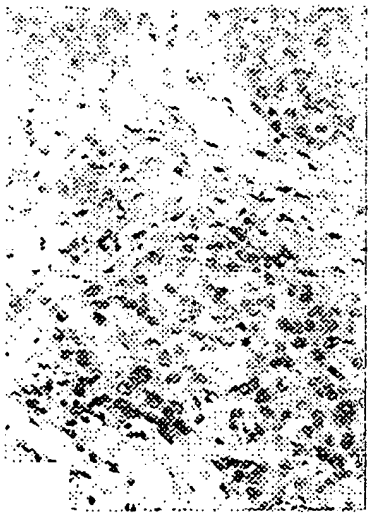


FIG.2F

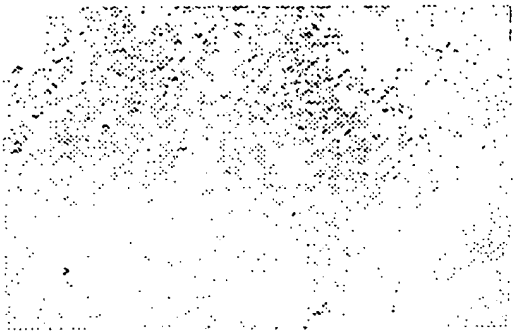
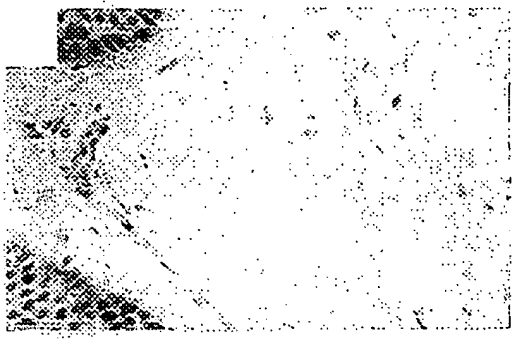


FIG.2G



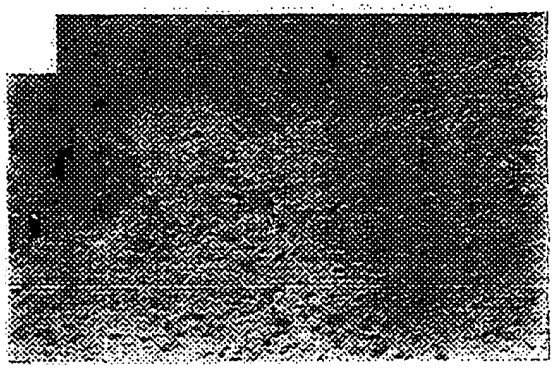


FIG.2H

```
AGACAAGGCA GGGGACGCAC CAAGG ATG GAG ATG TTC CAG GGG CTG CTG CTG    52
                            Met Glu Met Phe Gln Gly Leu Leu Leu
                           -20                 -15
TTG CTG CTG CTG AGC ATG GGC GGG ACA TGG GCA TCC AAG GAG CCG CTT   100
Leu Leu Leu Leu Ser Met Gly Gly Thr Trp Ala Ser Lys Glu Pro Leu
    -10                 -5                  1                 5
CGG CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG   148
Arg Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu
                10                  15                  20
GGC TGC CCC GTG TGC ATC ACC GTC AAC ACC ACC ATC TGT GCC GGC TAC   196
Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala GLy Tyr
            25                  30                  35
TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT   244
Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
        40                  45                  50
CAG CTG GTG TGC AAC TAC CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC   292
Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu
    55                  60                  65
CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT   340
Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala
70                  75                  80                  85
CTC AGC TGT CAA TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG   388
Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser THr Thr Asp Cys Gly
                90                  95                  100
GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC   436
Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp
            105                 110                 115
TCC TCT TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA   484
Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg
        120                 125                 130
CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAAAGGCTTC        530
Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
    135                 140                 145
TCAATCCGC                                                         539
```

FIG.3

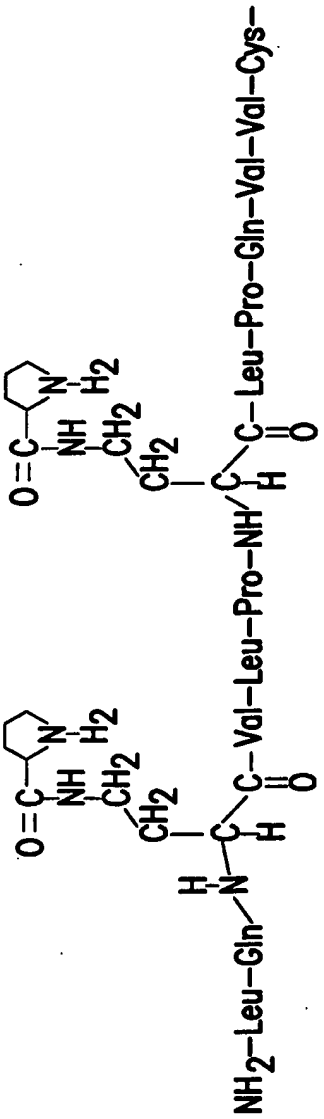

NH2–Leu–Gln–Val–Leu–Pro–NH–Leu–Pro–Gln–Val–Val–Cys–

FIG.4A

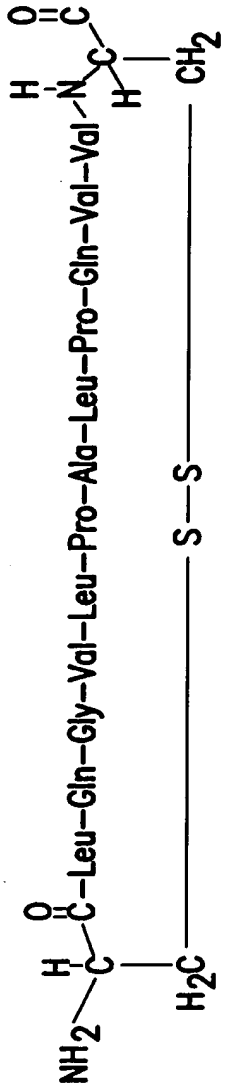

Leu–Gln–Gly–Val–Leu–Pro–Ala–Leu–Pro–Gln–Val–Val

FIG.4B

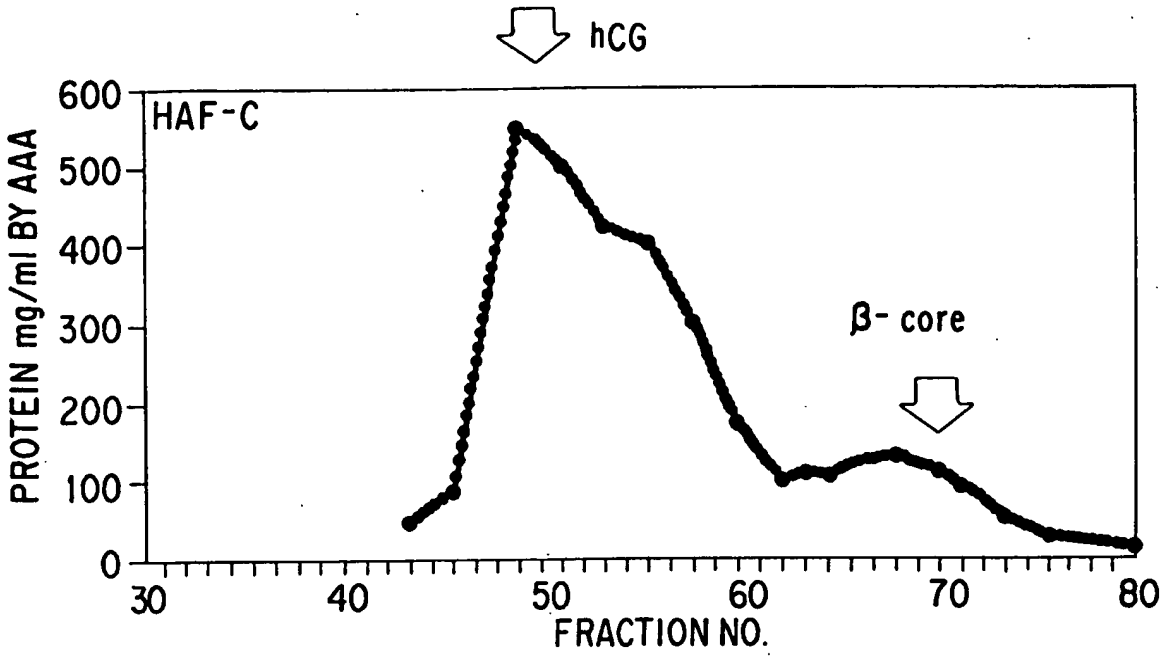

hCG
HAF-C
β- core
PROTEIN mg/ml BY AAA
600
500
400
300
200
100
0
30
40
50
60
70
80
FRACTION NO.

FIG. 5A

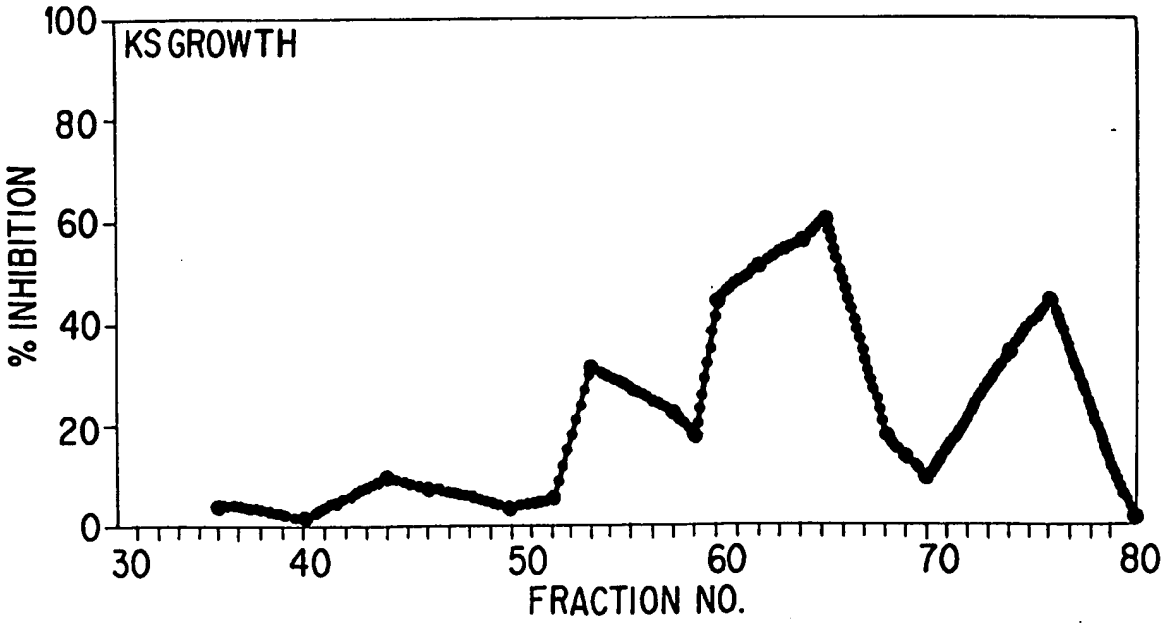

KS GROWTH
% INHIBITION
100
80
60
40
20
0
30
40
50
60
70
80
FRACTION NO.

FIG. 5B

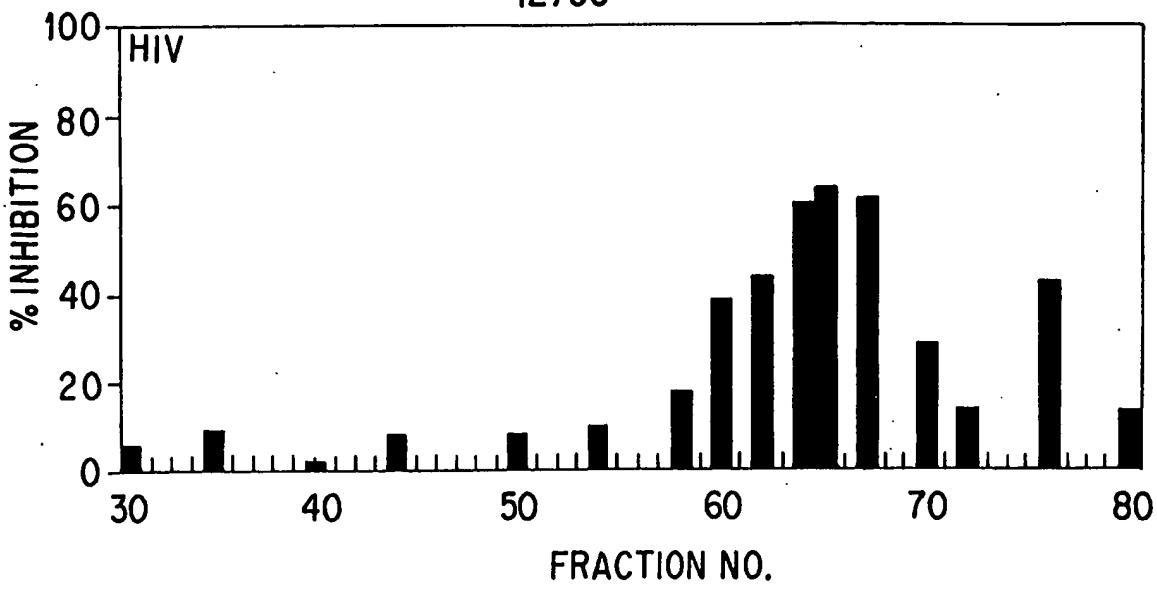

HIV
% INHIBITION
100
80
60
40
20
0
30
40
50
60
70
80
FRACTION NO.

FIG. 5C

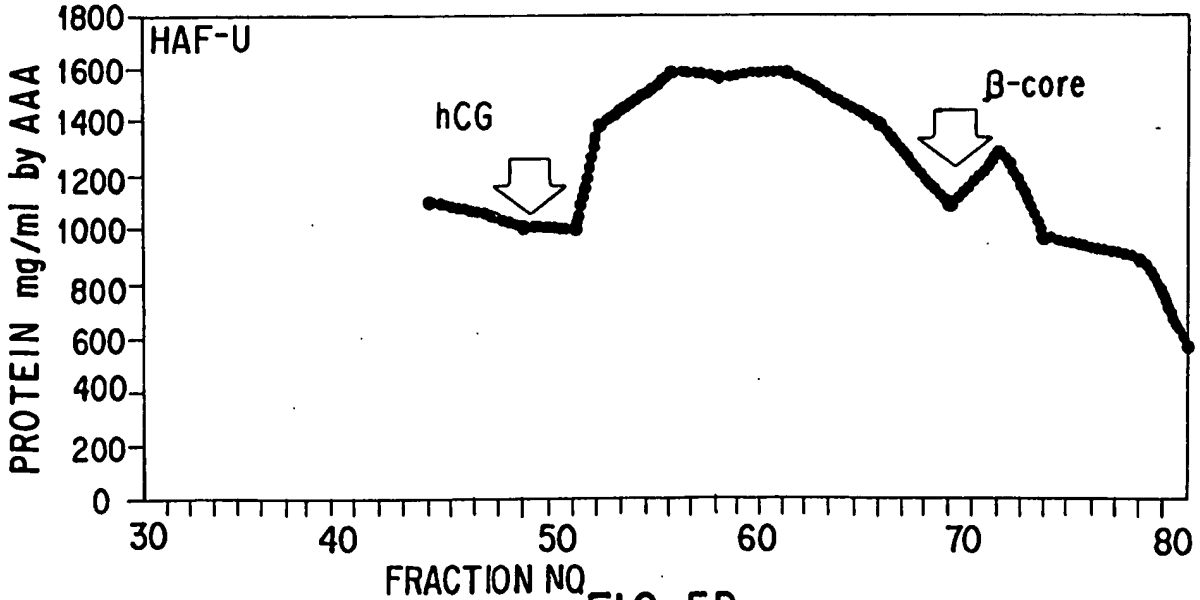

HAF-U
PROTEIN mg/ml by AAA
1800
1600
1400
1200
1000
800
600
400
200
0
hCG
β-core
30
40
50
60
70
80
FRACTION NO.

FIG. 5D

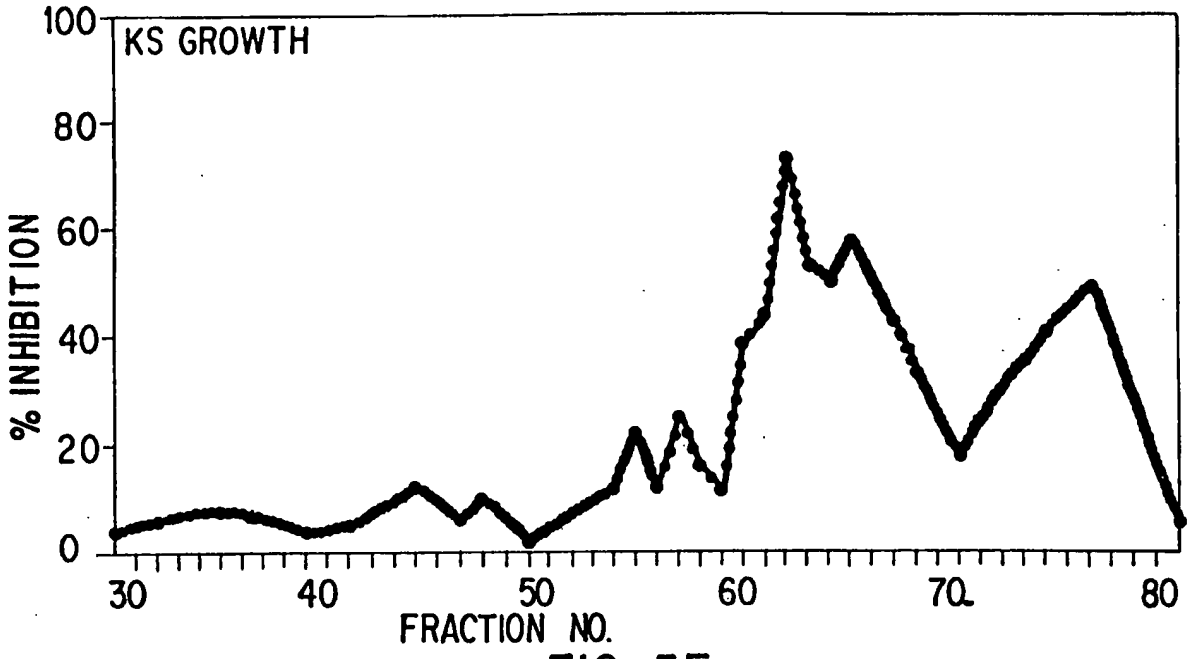

KS GROWTH
% INHIBITION
100
80
60
40
20
0
30
40
50
60
70
80
FRACTION NO.

FIG. 5E

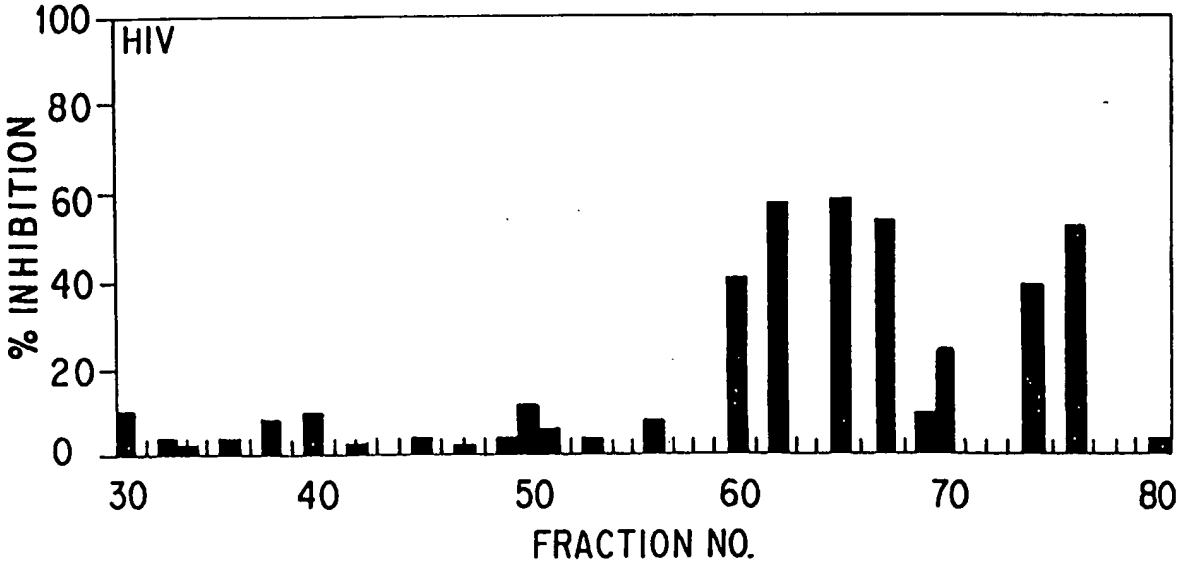

HIV
% INHIBITION
100
80
60
40
20
0
30
40
50
60
70
80
FRACTION NO.

FIG. 5F

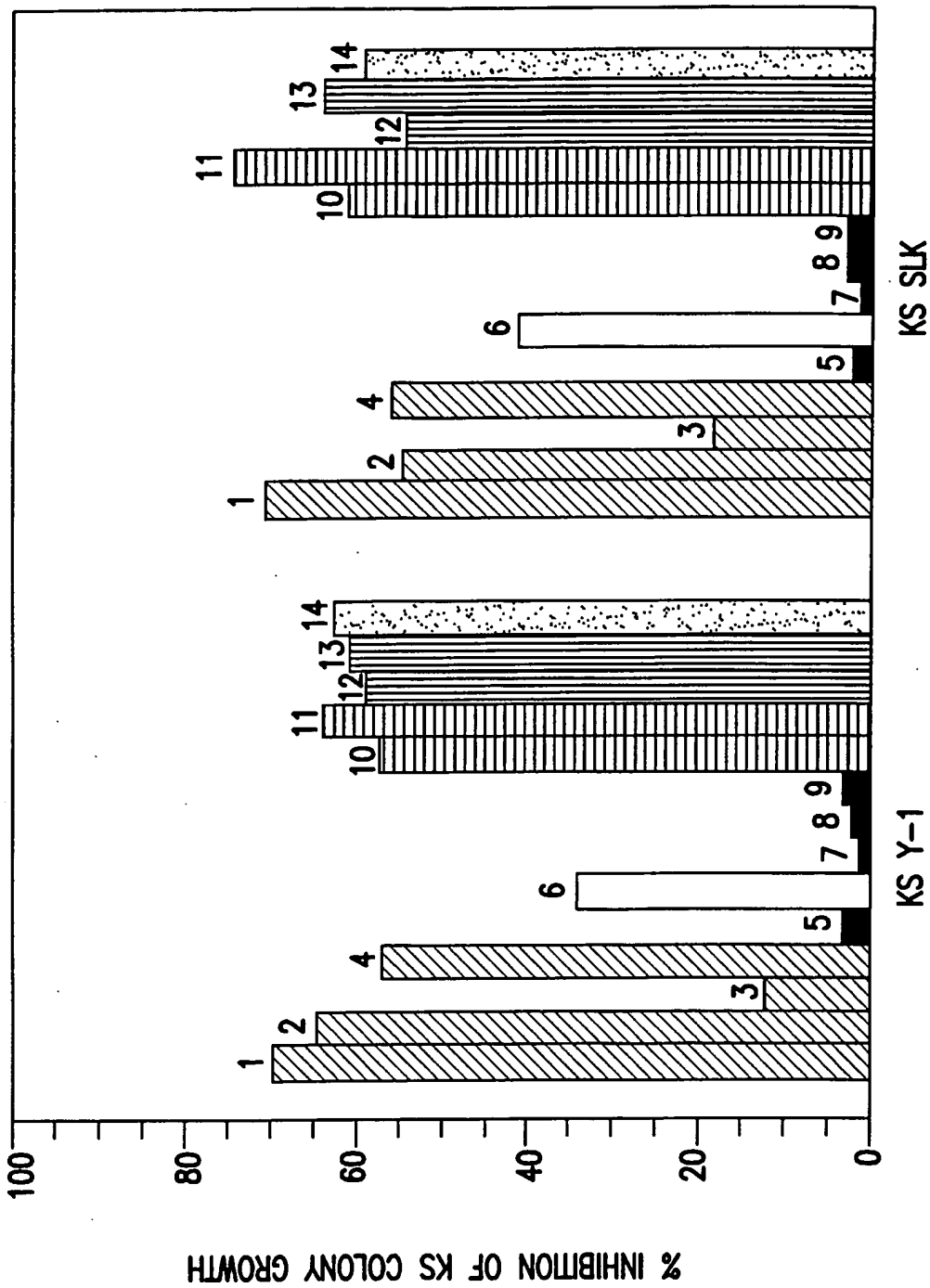

% INHIBITION OF KS COLONY GROWTH
100
80
60
40
20
0
KS Y-1
KS SLK
1
2
3
4
5
6
7
8
9
10
11
12
13
14

FIG.6

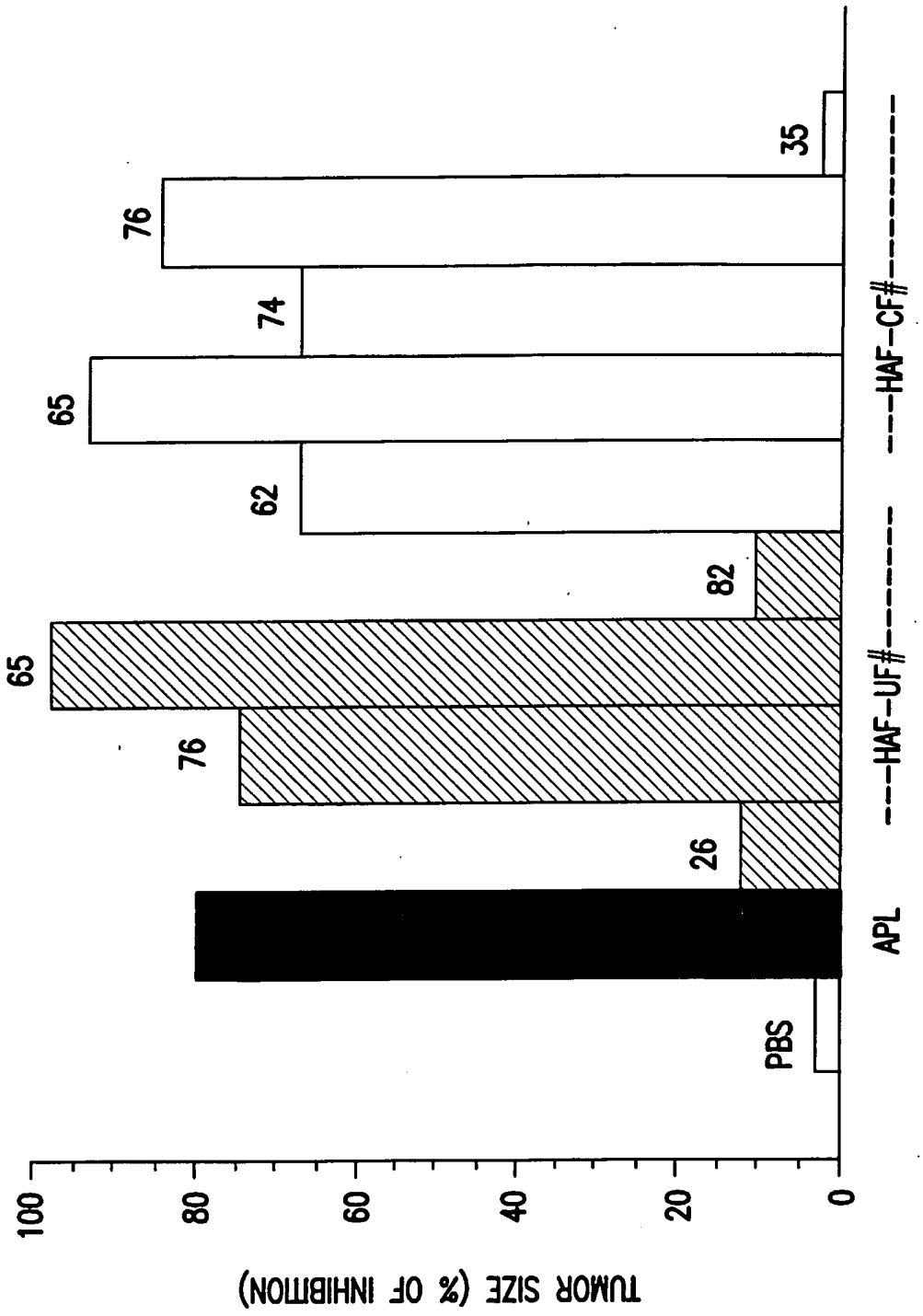

TUMOR SIZE (% OF INHIBITION)
100
80
60
40
20
0
PBS
APL
26
76
65
82
62
65
74
76
35
---HAF-UF#---
---HAF-CF#---
TREATMENT

FIG.7

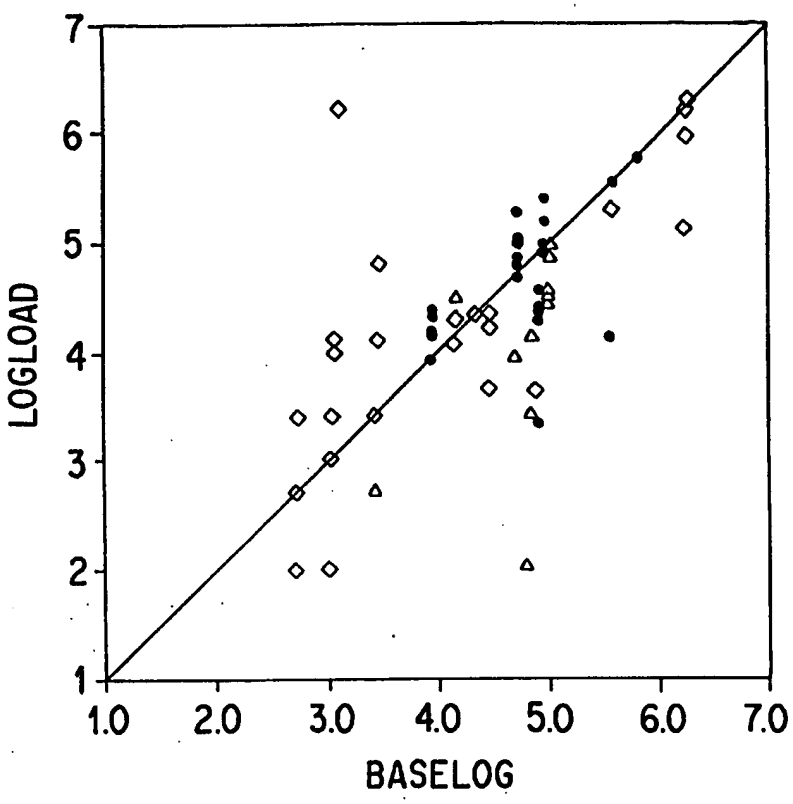
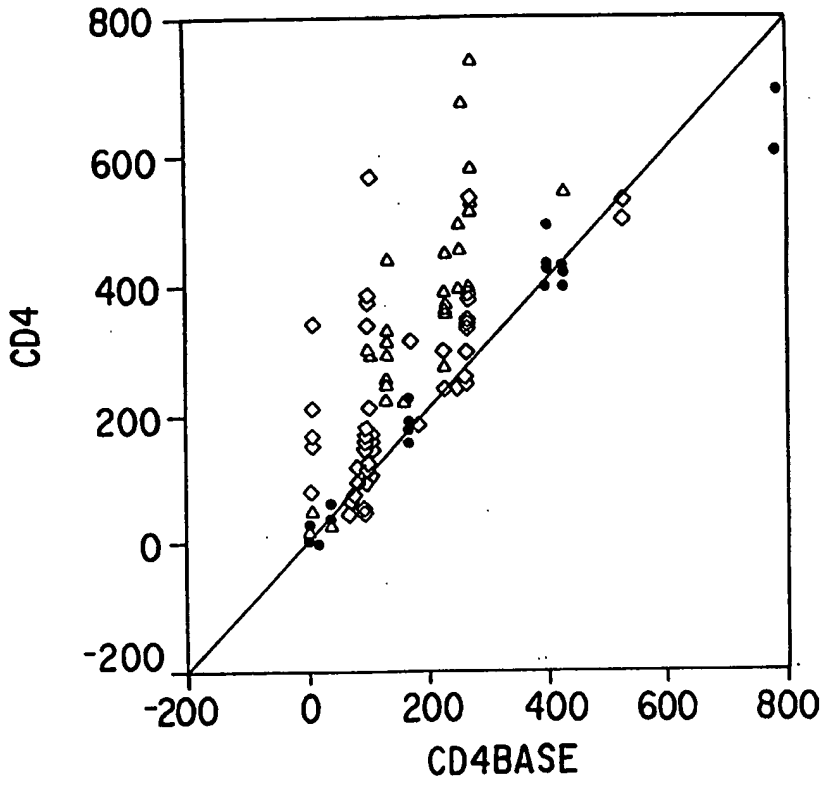

LOGLOAD
7
6
5
4
3
2
1
1.0
2.0
3.0
4.0
5.0
6.0
7.0
BASELOG
Rx : △ NON-PROTEASE & PROTEASE
◇ NON-PROTEASE
• hCG ALONE

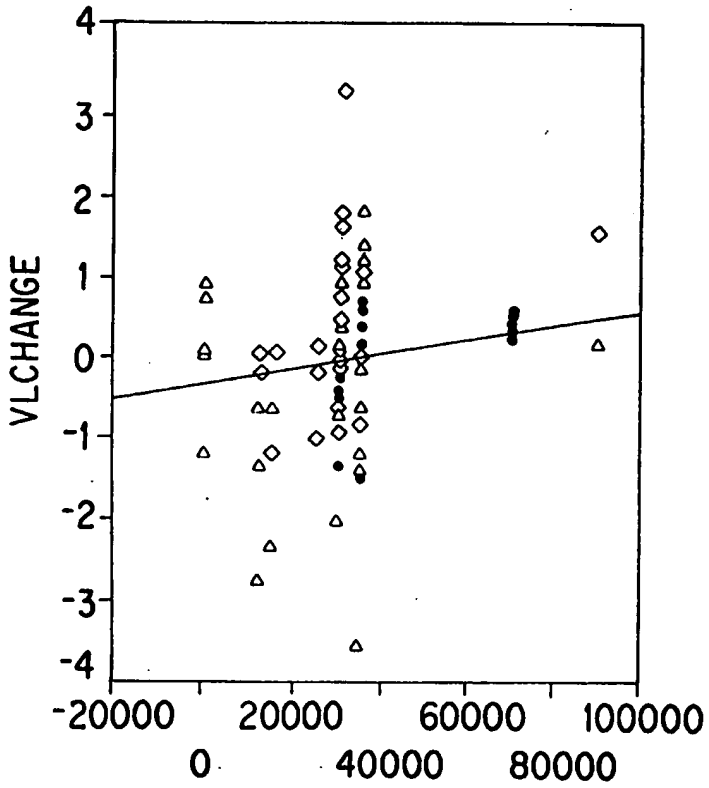
FIG. 8A



R_x: △ NON-PROTEASE & PROTEASE △ NON-PROTEASE

• hCG ALONE

FIG. 8B



RX: △ NON-PROTEASE & PROTEASE ◇ NON-PROTEASE

• hCG ALONE —TOTAL POPULATION

FIG. 8C

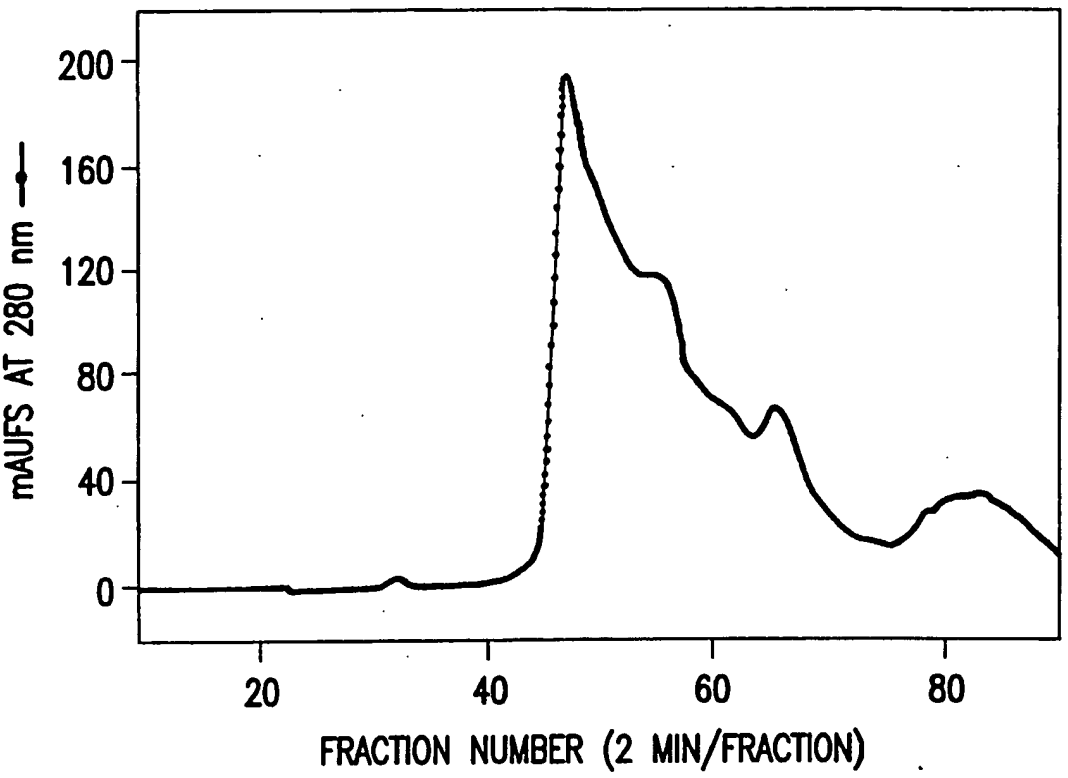

200
160
120
80
40
0
mAUFS AT 280 nm
20
40
60
80
FRACTION NUMBER (2 MIN/FRACTION)

FIG.9A

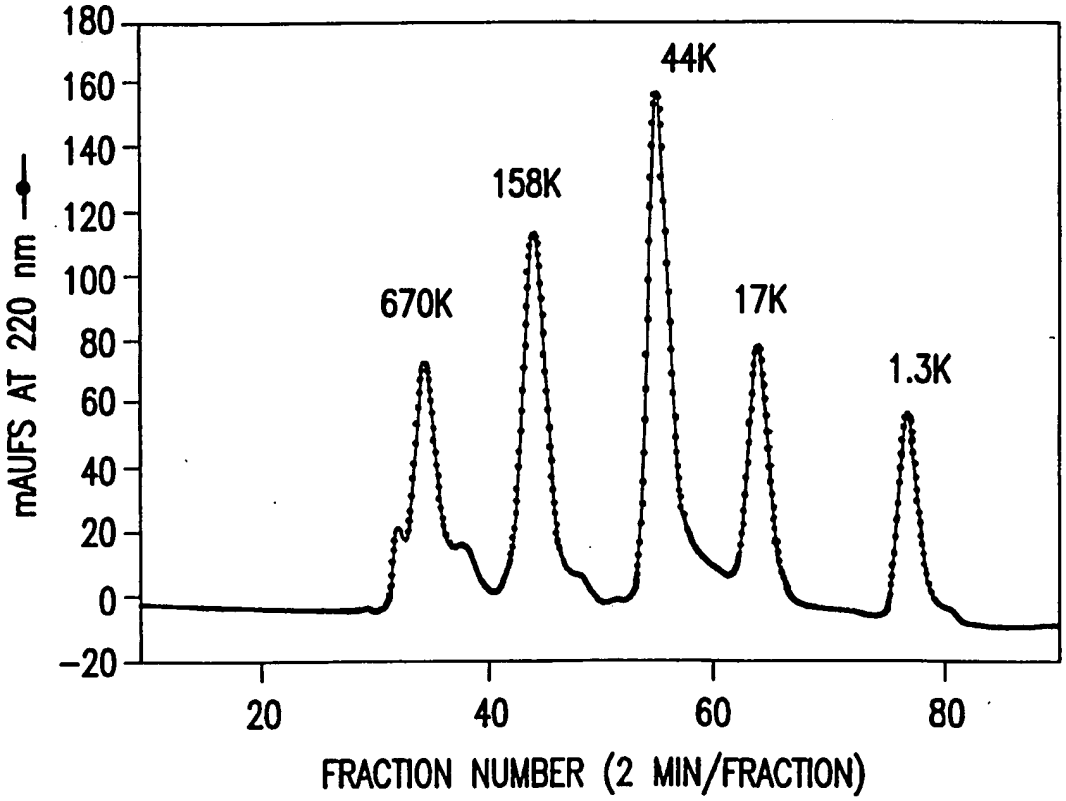

180
160
140
120
100
80
60
40
20
0
-20
mAUFS AT 220 nm
670K
158K
44K
17K
1.3K
20
40
60
80
FRACTION NUMBER (2 MIN/FRACTION)

FIG.9B

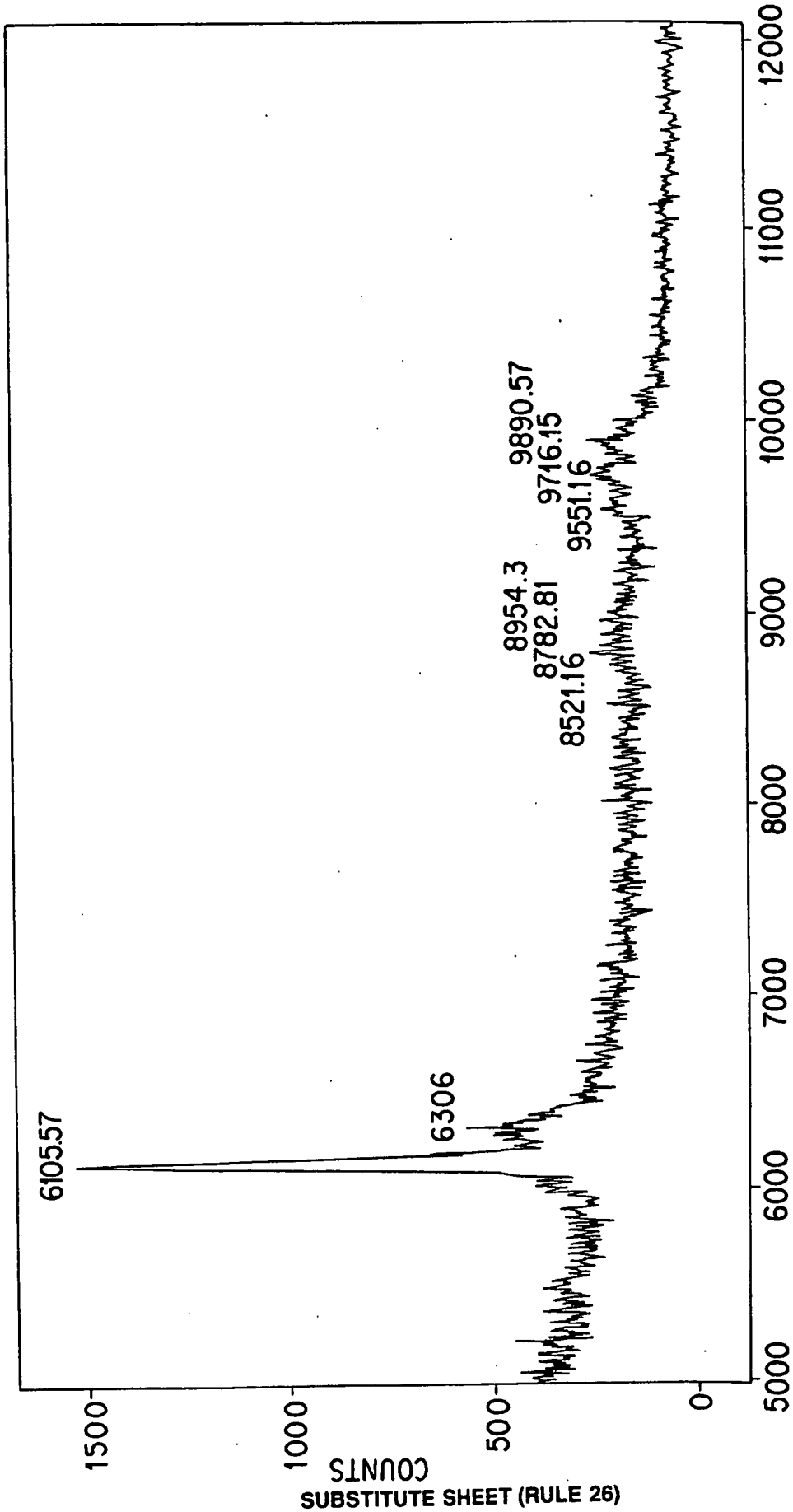

6105.57
6306
8521.16
8782.81
8954.3
9551.16
9716.15
9890.57
COUNTS
1500
1000
500
0
5000
6000
7000
8000
9000
10000
11000
12000
MASS(m/z)

FIG.10A

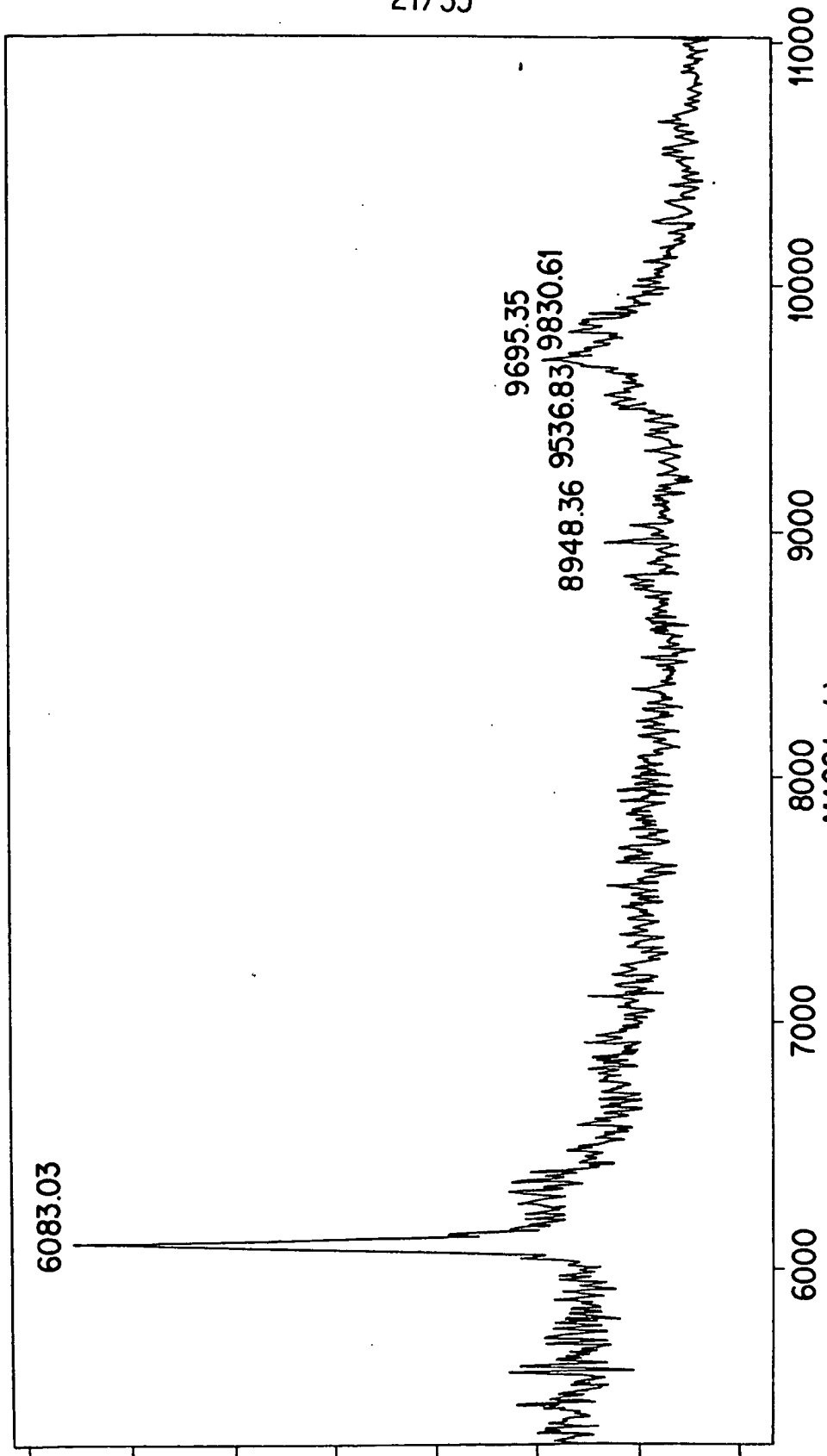

6083.03
9695.35
9830.61
9536.83
8948.36
COUNTS
1400
1200
1000
800
600
400
200
0
6000
7000
8000
9000
10000
11000
MASS (m/z)

FIG.10B

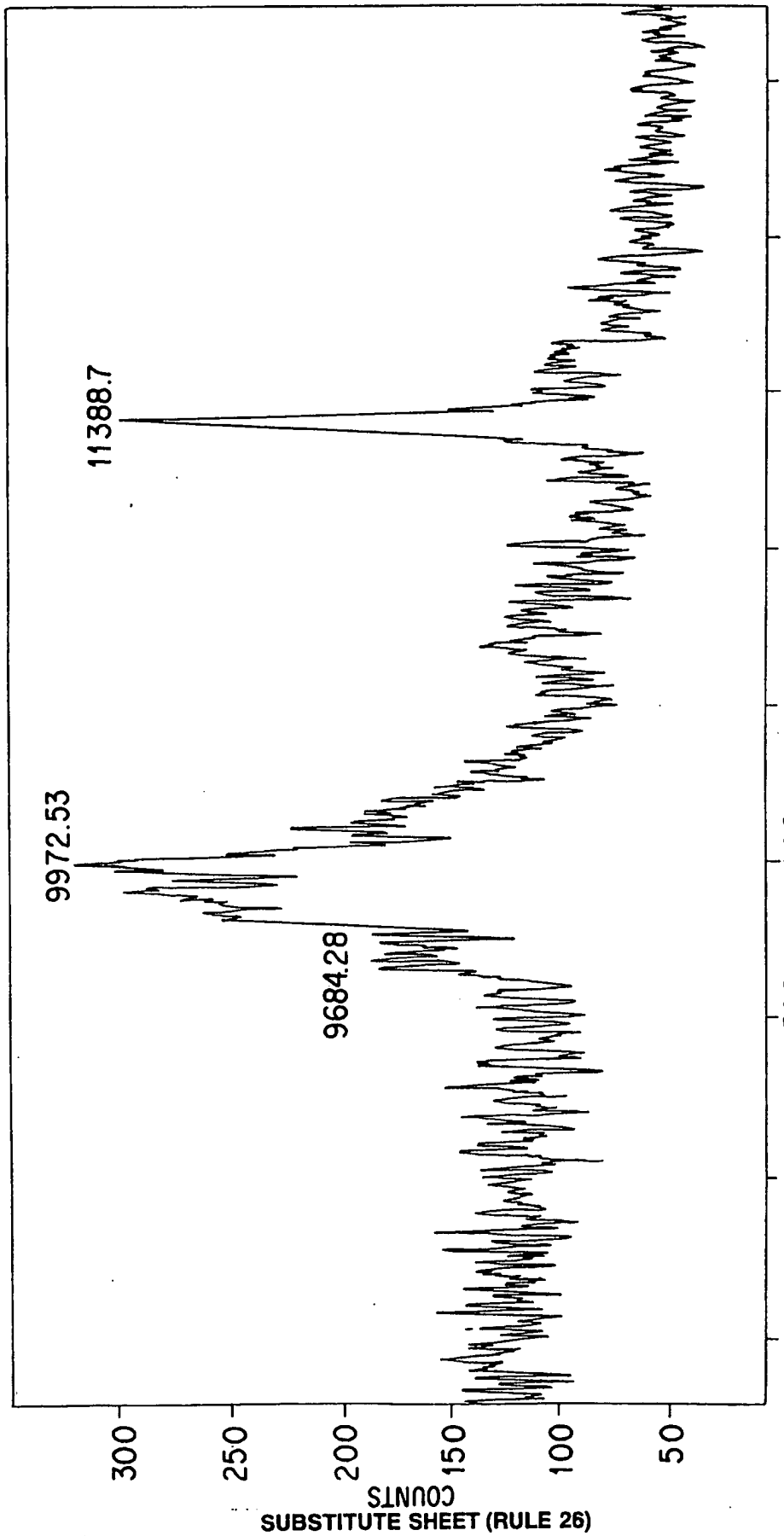

11388.7
9972.53
9684.28
COUNTS
300
250
200
150
100
50
8500
9000
9500
10000
10500
11000
11500
12000
12500
MASS(mz)

FIG.10C

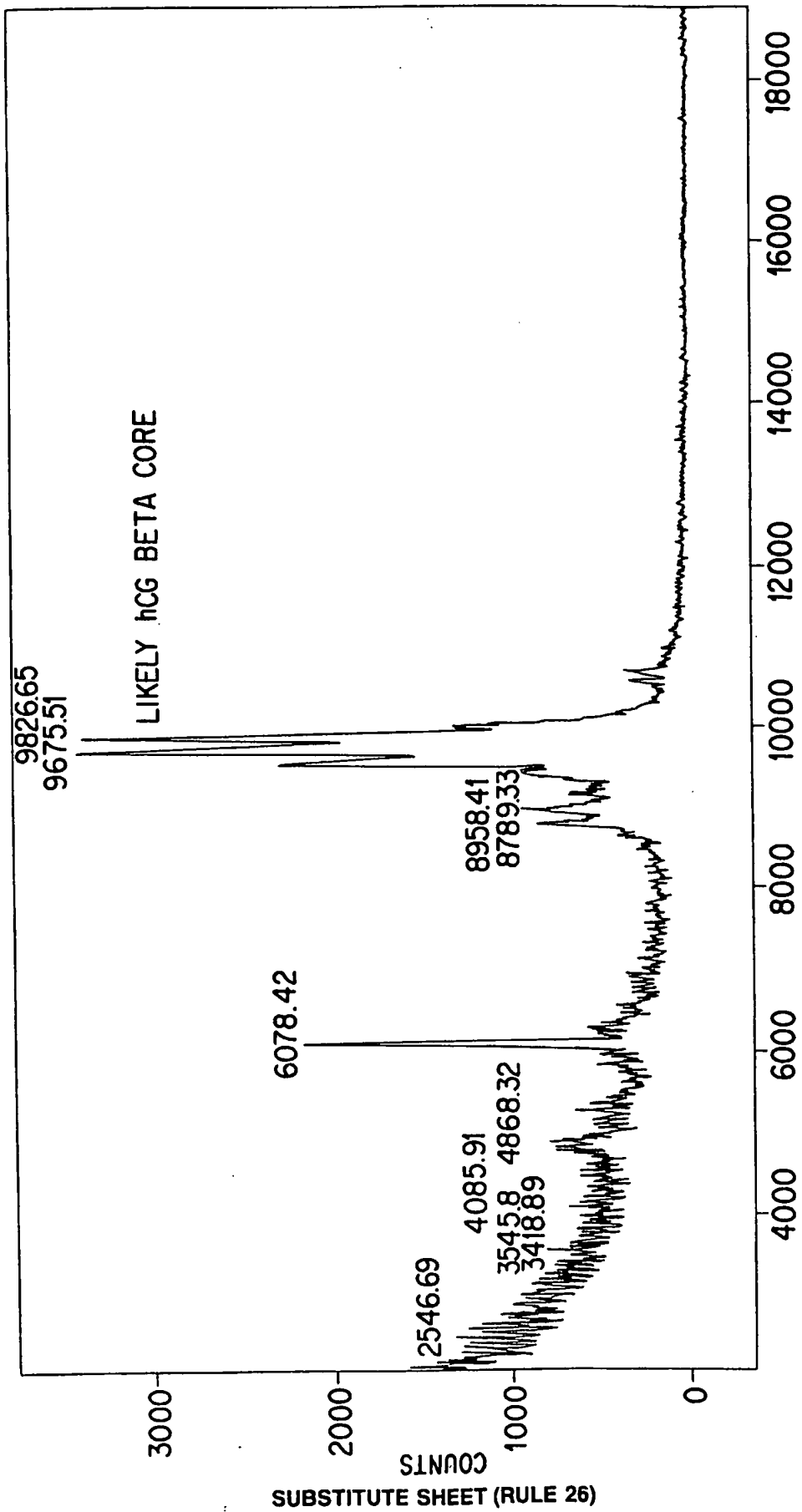

LIKELY hCG BETA CORE
9826.65
9675.51
8958.41
8789.33
6078.42
4868.32
4085.91
3545.8
3418.89
2546.69
COUNTS
MASS (m/z)
0
1000
2000
3000
4000
6000
8000
10000
12000
14000
16000
18000

FIG.10D

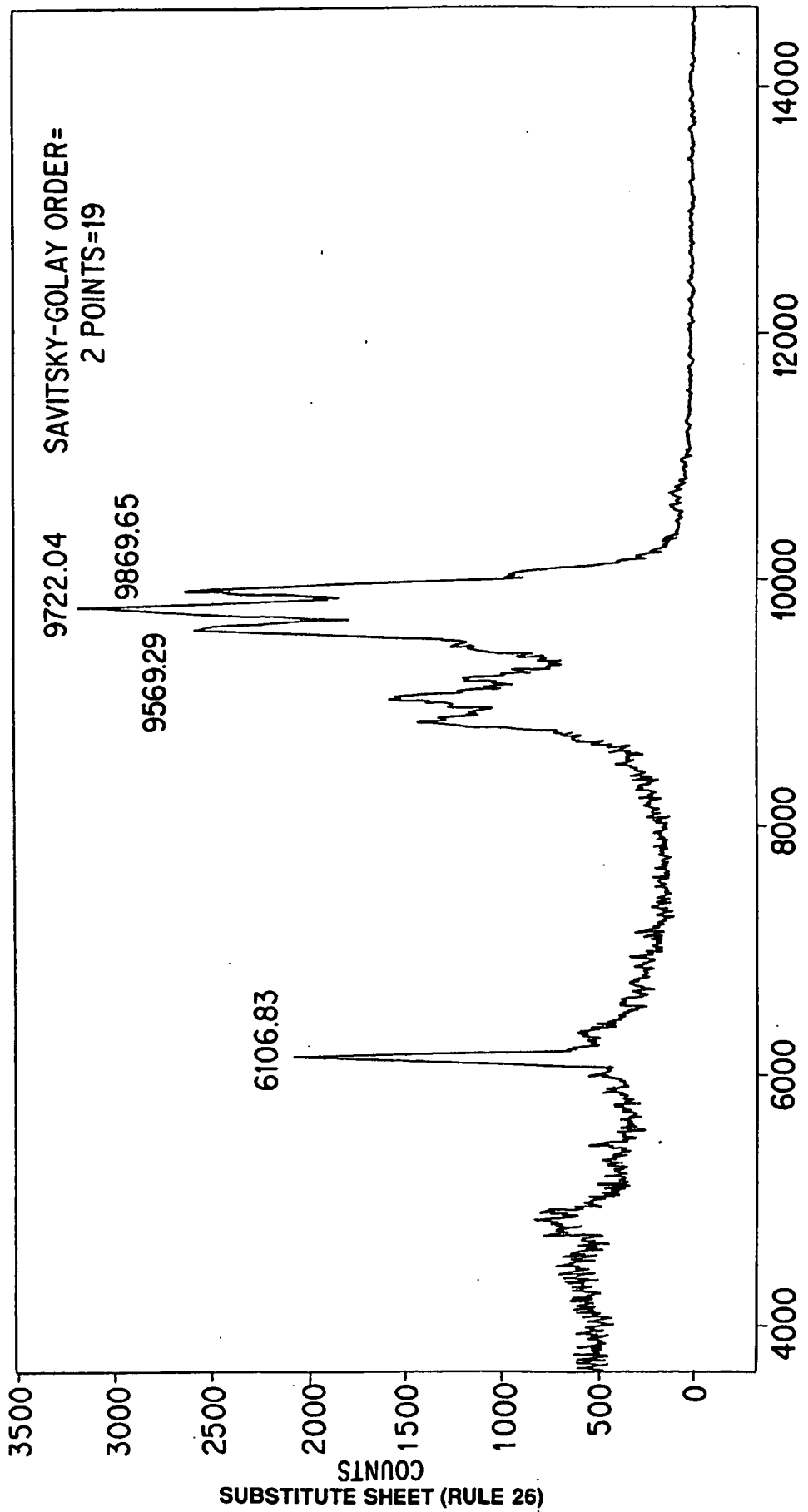

SAVITSKY-GOLAY ORDER=
2 POINTS=19
9722.04
9869.65
9569.29
6106.83
COUNTS
MASS (m/z)
0
500
1000
1500
2000
2500
3000
3500
4000
6000
8000
10000
12000
14000

FIG.10E

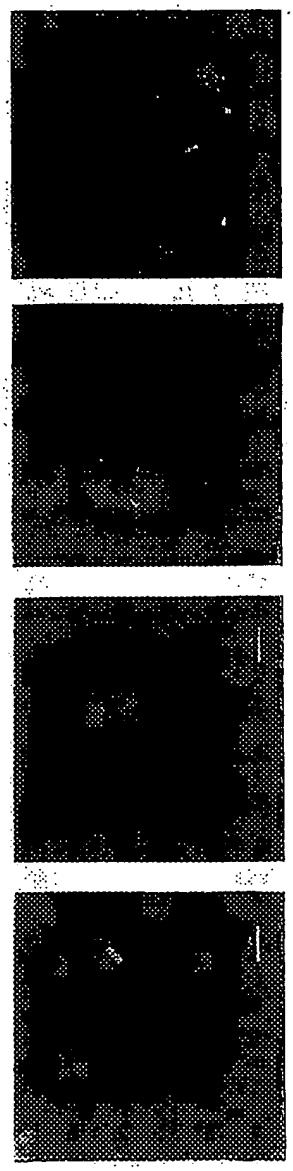


FIG.11A FIG.11B FIG.11C FIG.11D

FIG.11E FIG.11F FIG.11G FIG.11H

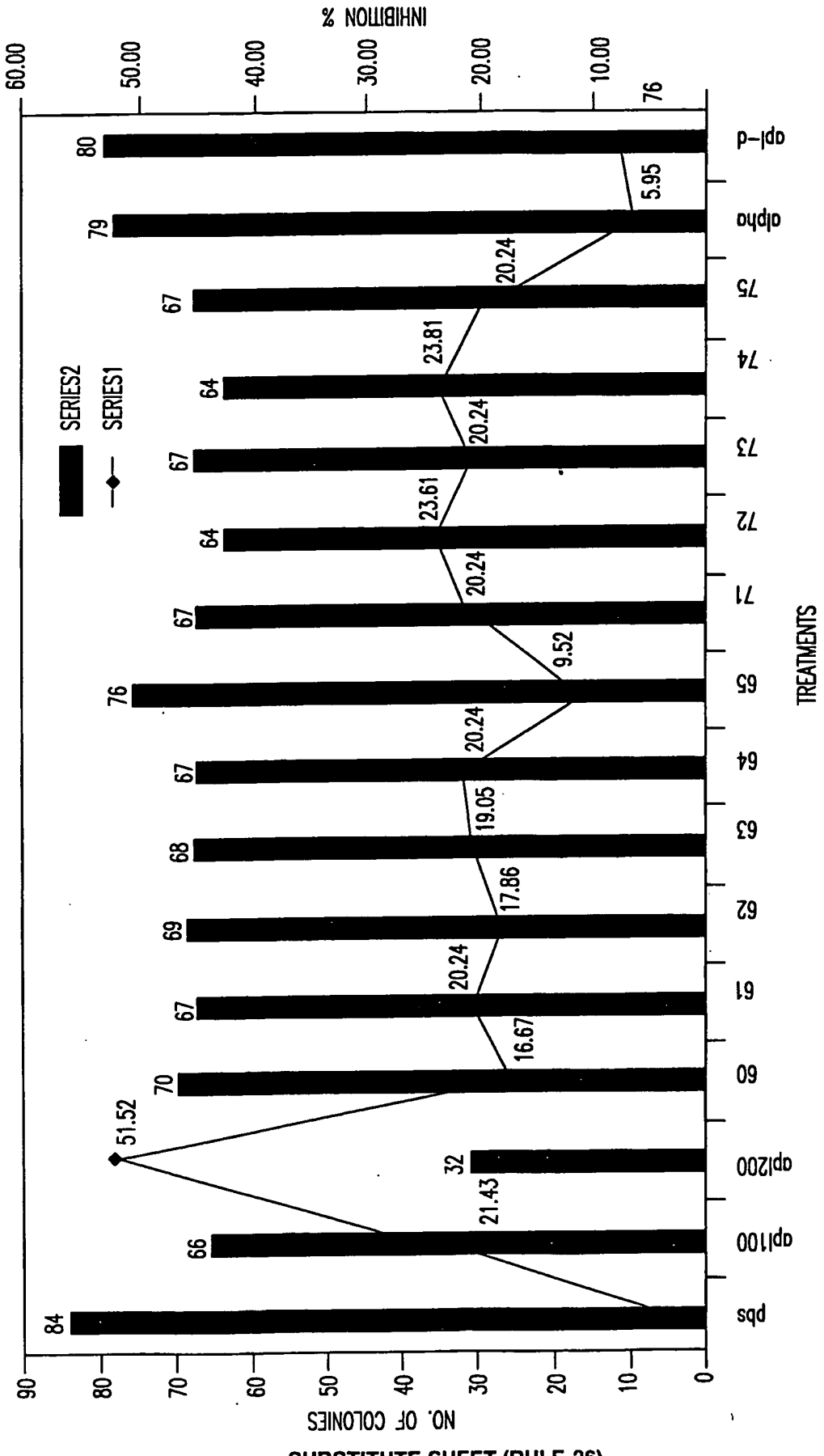

INHIBITION %
60.00
50.00
40.00
30.00
20.00
10.00
76
SERIES2
SERIES1
80
79
67
64
67
64
67
76
67
68
69
67
70
32
66
84
5.95
20.24
23.81
20.24
23.61
20.24
9.52
20.24
19.05
17.86
20.24
16.67
51.52
21.43
apl-d
alpha
75
74
73
72
71
65
64
63
62
61
60
apl200
apl100
pbs
TREATMENTS
NO. OF COLONIES
90
80
70
60
50
40
30
20
10
0

FIG.12

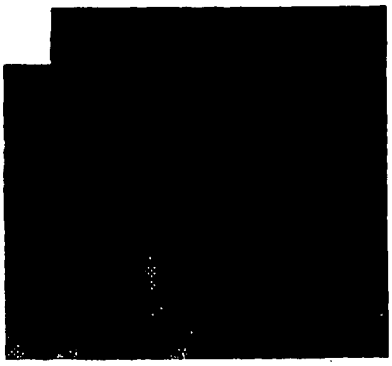


FIG.13A

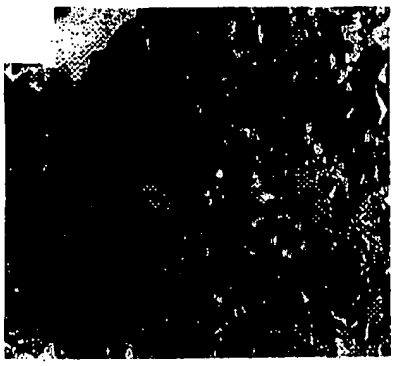


FIG.13B

FIG.13C

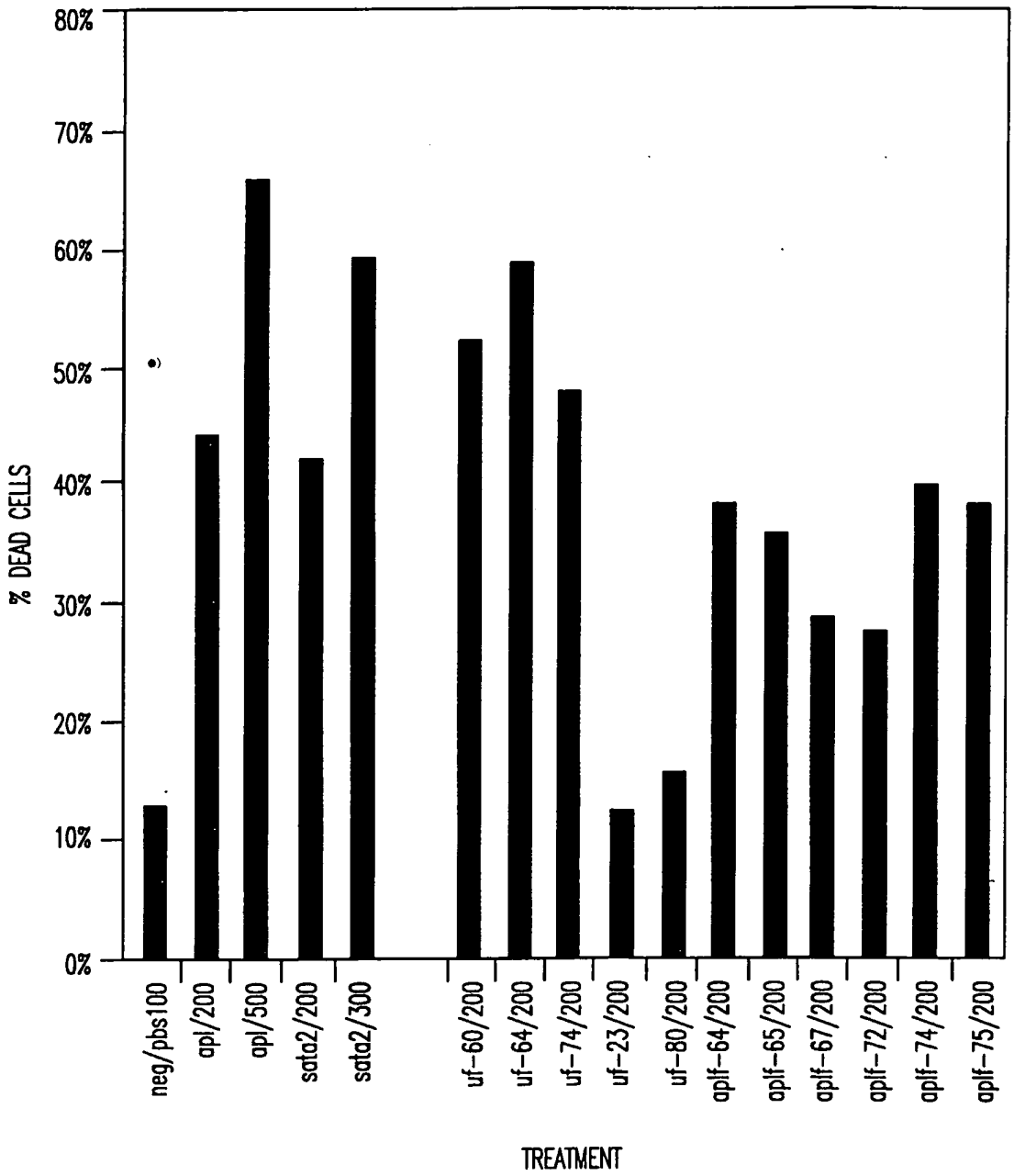

80%
70%
60%
50%
40%
30%
20%
10%
0%
% DEAD CELLS
neg/pbs100
apl/200
apl/500
sata2/200
sata2/300
uf-60/200
uf-64/200
uf-74/200
uf-23/200
uf-80/200
aplf-64/200
aplf-65/200
aplf-67/200
aplf-72/200
aplf-74/200
aplf-75/200
TREATMENT

FIG.14

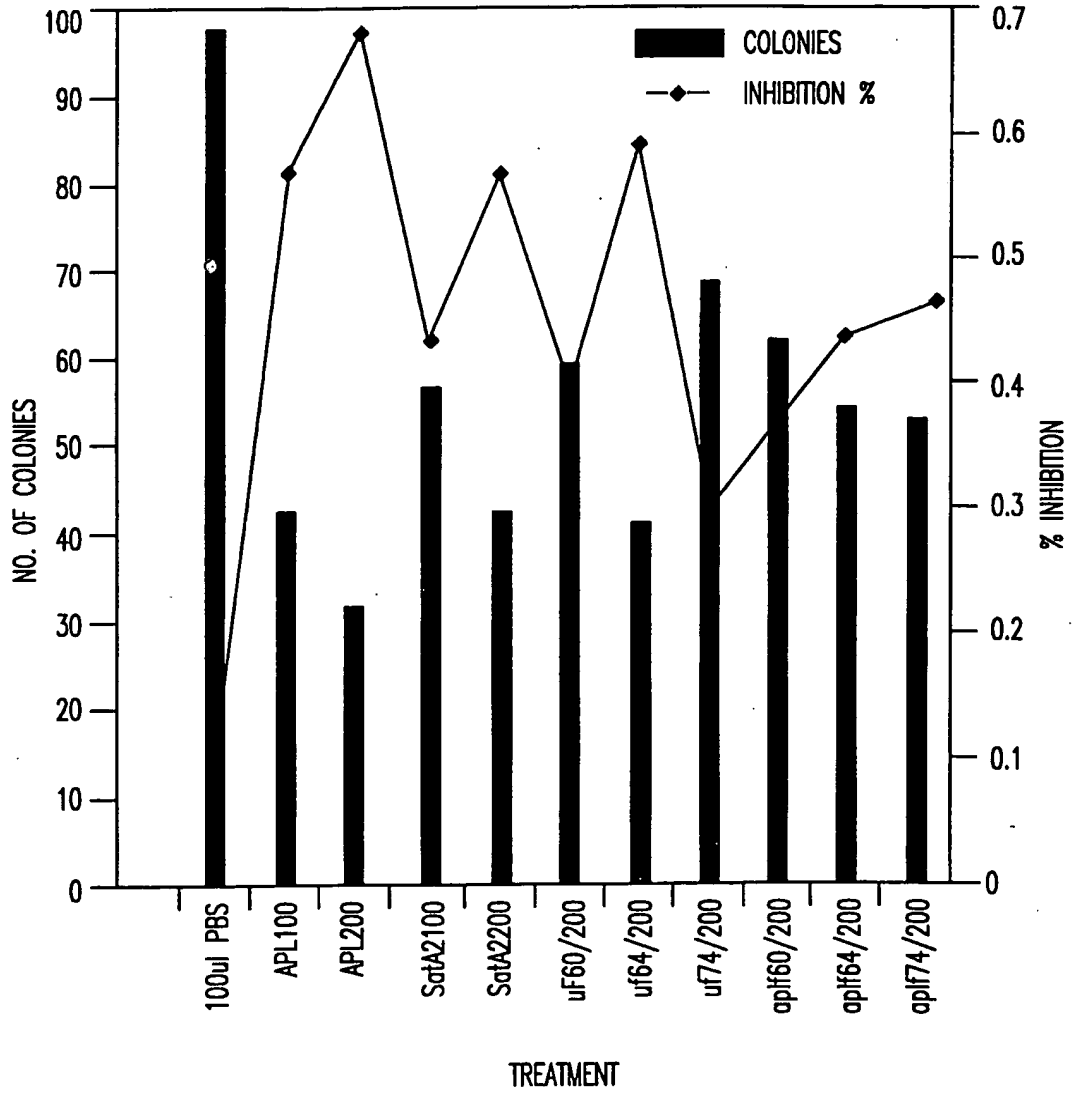

COLONIES
INHIBITION %
NO. OF COLONIES
% INHIBITION
0
10
20
30
40
50
60
70
80
90
100
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
100ul PBS
APL100
APL200
SatA2100
SatA2200
uF60/200
uf64/200
uf74/200
aplf60/200
aplf64/200
aplf74/200
TREATMENT

FIG.15A

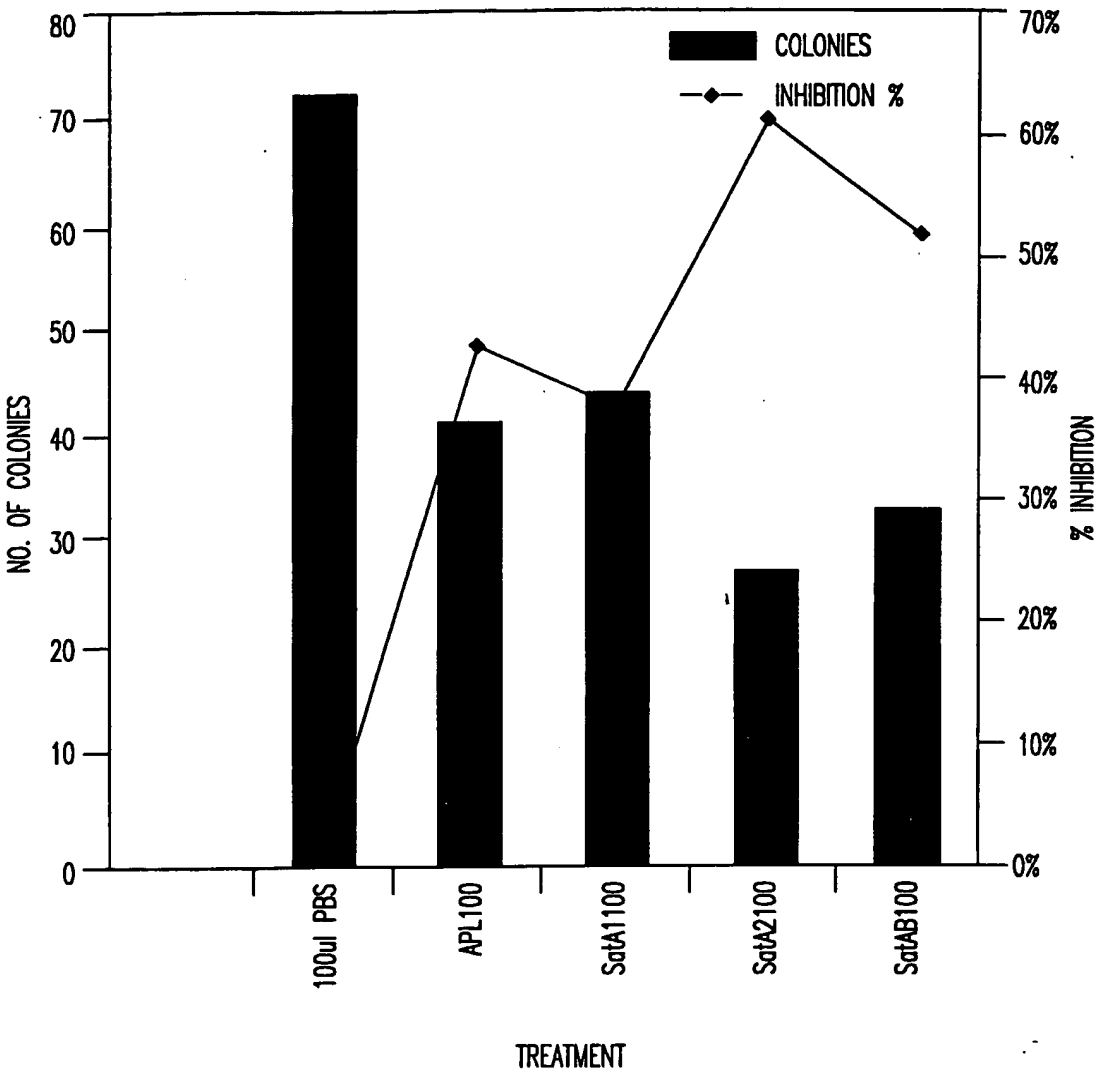

80
70
60
50
40
30
20
10
0
NO. OF COLONIES
70%
60%
50%
40%
30%
20%
10%
0%
% INHIBITION
COLONIES
INHIBITION %
100ul PBS
APL100
SatA1100
SatA2100
SatAB100
TREATMENT

FIG.15B

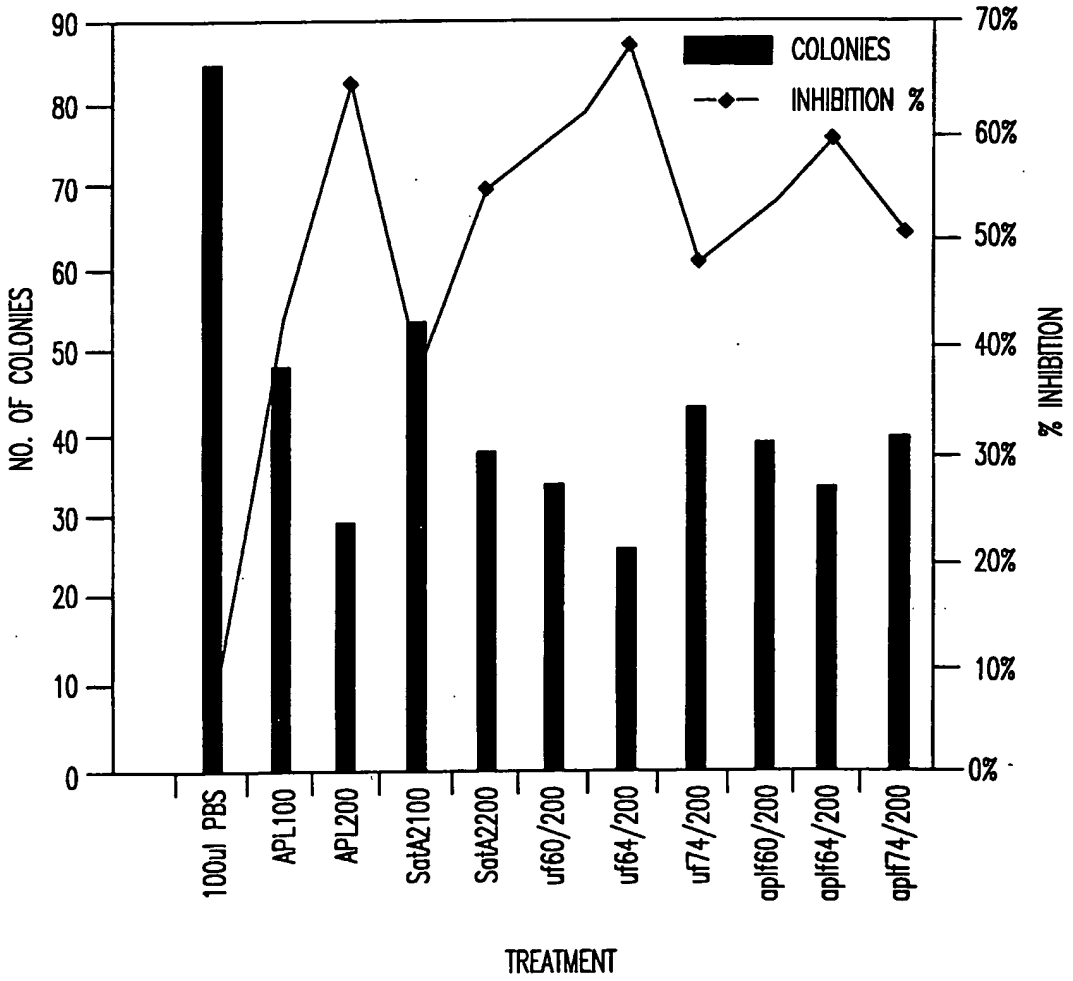

90
80
70
60
50
40
30
20
10
0
NO. OF COLONIES
70%
60%
50%
40%
30%
20%
10%
0%
% INHIBITION
COLONIES
INHIBITION %
100ul PBS
APL100
APL200
SatA2100
SatA2200
uf60/200
uf64/200
uf74/200
aplf60/200
aplf64/200
aplf74/200
TREATMENT

FIG.15C

FIG.16C

FIG.16B

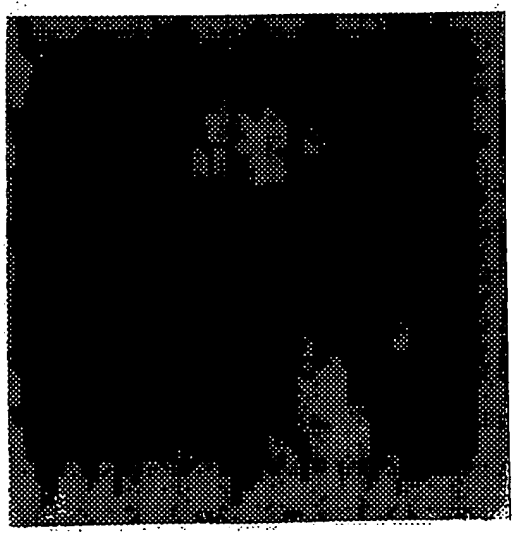


FIG.16A

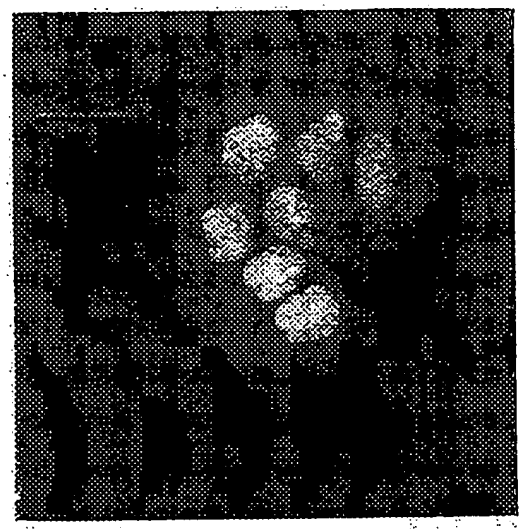


FIG.16F

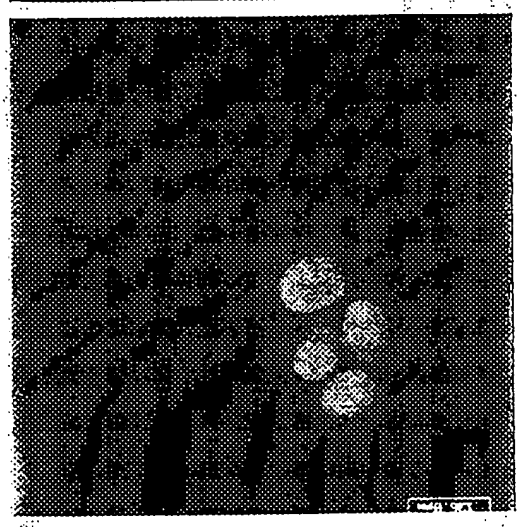


FIG.16E

FIG.16D

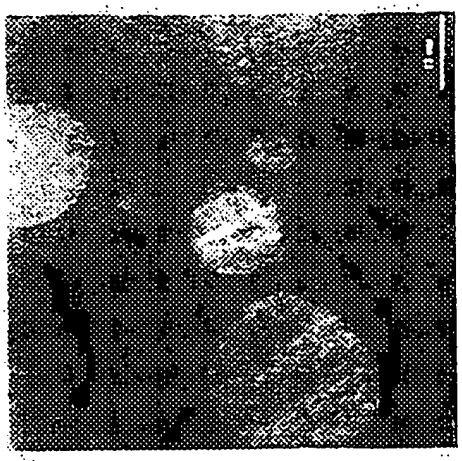


FIG.17C

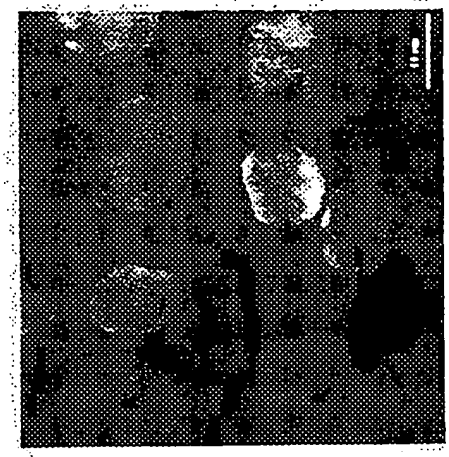


FIG.17F

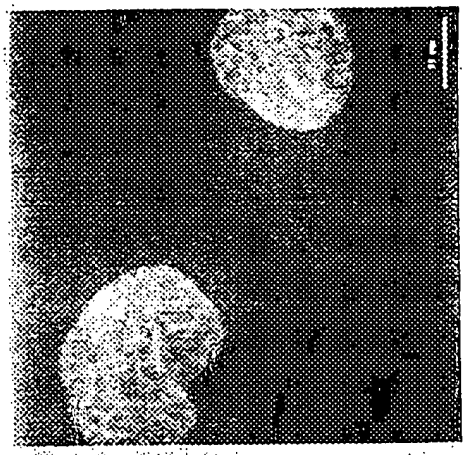


FIG.17B

FIG.17E

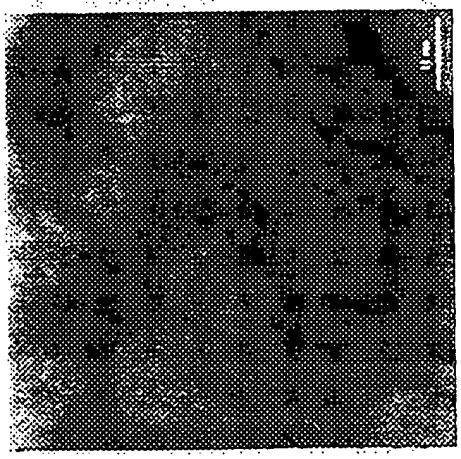


FIG.17A

FIG.17D

FIG. 17I

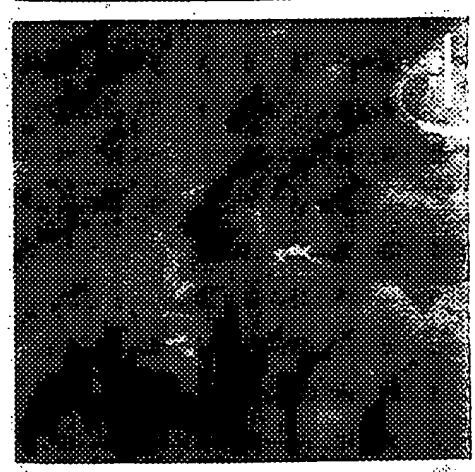


FIG. 17H

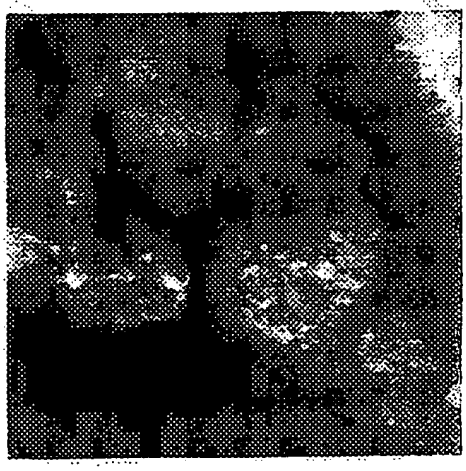


FIG. 17G

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/11210

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00, 39/00, 38/00, 31/70; A01N 43/04, 63/00; G01N 33/574
US CL : 514/11, 12, 44; 435/7.23; 424/9.2, 93.2, 185.1, 198.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/11, 12, 44; 435/7.23; 424/9.2, 93.2, 185.1, 198.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, Biosis, Embase, CancerLit, Scisearch, WPIDS
search terms: Human chorionic gonadotropin, peptides, cancer treatment

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KEUTMANN et al. A Receptor-Binding Region in Human Choriogonadotropin/lutropin β subunit. Proc. Natl. Acad. Sci. USA. April 1987, Vol. 84, pages 2038-2042, see especially Methods.	1, 5, 9, 11-18, 23 and 26-28
Y	US 4,161,519 A (TALWAR) 17 July 1979, columns 5-6.	43, 95-101
Y	US 4,780,312 A (TALWAR) 25 October 1988, whole article.	1, 5, 9, 11-18, 23, 26-28, 38-43, and 95-101
Y	US 4,767,842 A (STEVENS) 30 August 1988, whole article.	1, 5, 9, 11-18, 23, 26-28, and 44-52

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
14 SEPTEMBER 1997

Date of mailing of the international search report
24 OCT 1997

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/11210

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1, 5, 9, 11-18, 23 and 26-28, drawn to a method of treatment using peptides of HCG.

Group II, claims 38-43, drawn to drawn to a method of treatment using HCG purified from a mixture.

Group III, claims 44-52, drawn to a method of screening.

Group IV, claims 95-101, drawn to a composition HCG purified from a mixture.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

Species I is drawn to linear peptides and derivatives.
Species II is drawn to circularized peptides.
Species III is drawn to branched chain peptides.

The claims are deemed to correspond to the species listed above in the following manner:

Species I- claims 1, 5, 9, and 11-17.
Species II- claims 18 and 28.
Species III- claim 23.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical features of the different groups do not share a single inventive concept. The special technical feature of Group II and Group IV are drawn to purified HCG, but the two groups are distinct as Group II relates to a method of treatment and Group IV to a composition. The special technical feature of Group III is not found in the claims of Groups I, II, and IV and the special technical features of Groups I, II, and IV are not found in the claims of Group III.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Species I-III are not unified by a special technical feature, because the different species are structurally and chemically different and have different process steps to produce them.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.